KU LEUVEN

ARENBERG DOCTORAL SCHOOL
FACULTY OF SCIENCE

Integrating trait and phylogenetic distances in metacommunity analysis and ecosystem functioning research

Andros Gianuca

Promotor:
Prof. Dr Luc De Meester

Dissertation presented in partial fulfilment of the requirements for the degree of Doctor in Science (Biology)

January 2017

Science, Engineering and Technology Group
Faculty of Science
Department of Biology
Ecology, Evolution and Biodiversity Conservation Section

Laboratory of Aquatic Ecology, Evolution and Conservation

Ch. Deberiotstraat 32

B-3000 Leuven

KATHOLIEKE
UNIVERSITEIT
LEUVEN

Andros GIANUCA

Supervisor:

Prof. dr Luc De Meester
Laboratory of Aquatic Ecology, Evolution and Conservation, K.U.Leuven, Leuven, Belgium

Members of the Examination Committee:

Prof. dr Ellen Decaestecker
Laboratory of Aquatic Biology, K.U.Leuven Kulak, Kortrijk, Belgium

Prof. dr Dries Bonte
Terrestrial Ecology Unit, Department of Biology, Ghent University, Ghent, Belgium

Prof. dr Filip Volckaert
Laboratory of Biodiversity and Evolutionary Genomics, K.U.Leuven, Leuven, Belgium

Dr Steven Declerck
Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

Prof. dr Bram Vanschoenwinkel
Laboratory of Community Ecology, Vrije Universiteit Brussel, Brussels, Belgium

Dr Pieter Lemmens
Laboratory of Aquatic Ecology, Evolution and Conservation, K.U.Leuven, Leuven, Belgium

The research presented in this dissertation was funded by the Brazilian government through CNPq via a “Science Without Borders” full PhD scholarship to the author.

Uitgegeven in eigen beheer, Andros T. Gianuca, Leuven

Cover image portrays a metacommunity in the Pantanal biome, considered the largest freshwater wetland in the world and located in Brazil. Photography taken by: Valdemir Cunha/ Brasil Natural. Saved from google.com.

Contents

DEDICATION

I dedicate this thesis to my father, Norton Gianuca, who has been a source of inspiration in all aspects of my personal and professional development. I also dedicate this thesis to my wife, Monica Veloso, because her support, love and comprehension helped me throughout the whole process of my PhD formation.

ACKNOWLEDGEMENTS

I am first and foremost indebted to my supervisor Dr Luc De Meester, for his academic and moral support over the last four years. Dr Luc De Meester provided me the unique opportunity to join an excellent University and research team, an experience that has profoundly changed my perspective of science and that I will carry over with gratitude for the rest of my life. Dr Luc De Meester has not only offered me key counsel on how to write high-quality scientific articles but also on how to critically see through the lens of ecological and evolutionary theories.

I also would like to thank Dr Steven Declerck and Prof. Dr Marc Cadotte, who have inspired me from the very beginning of my studies in Ecology. Their crucial help and solicitude were not only relevant for the output of my research but also as a great example of humility and open mindness, which has influenced very positively my perspective of networking and my maturation as a scientist.

In addition, I would like to express my gratitude to the members of my examination committee Prof. Ellen Decaestecker, Prof. Dries Bonte, Dr Steven Declerck, Prof. Filip Volckaert, Dr Pieter Lemmens and Prof. Bram Vanschoenwinkel.

I am also grateful to all my colleagues who have shared with me many good moments during the last four years. I learnt a lot with you all: Adinda, Aline, Anurag, Arnout, Aurora, Carla, Caroline, Charlotte,

Conny, Eddy, Edwin, Eyerusalem, Falko, Fassil, Frans, Geert, Haile, Hendrik, Jasmien, Jelena, Jessie, Joost O, Joost R, Katina, Kjell, Koen, Kristien, Kuong, Laurens, Letícia Q., Lin, Lizanne, Lynn, Maarten, Marie, Matthias, Melissa, Mieke, Nedim, Pascal, Pieter, prof. Luc Brendonck, prof. Robby Stocks, Ria, Rony, Sabine, Sara D, Sarah O, Sophie-Delerue, Tom P, Toshiro, Trevor, Veerle, Xavier (in alphabetical order).

I would like to thank my family, especially my parents and my sister for all the support and good counsel.

Monica, my deepest and sincere thanks for all the support, love and comprehension!

Andros, January 2017

GENERAL INTRODUCTION

INTRODUCTION

1. Multidimensional biodiversity patterns across spatial scales.

Biodiversity is a multidimensional concept that can be quantified and interpreted as the variety of genes, species and/or functional traits in a given ecosystem (Cardinale et al. 2012, Swenson et al. 2012, Cadotte et al. 2013). Many studies have focused on the diversity of genes and functional traits at the population level (i.e., intra-specific trait variation), and have investigated the determinants and effects of population level diversity on ecosystem processes (McCauley et al. 1995, Hargrave et al. 2011, Perrier et al. 2011). Populations of different species often do not exist in isolation but rather co-occur and interact with populations of other species, forming the so-called ecological communities. At the community level, different dimensions of biodiversity can be similarly quantified. For instance, one can measure taxonomic diversity as the number of species within a community. Alternatively, functional trait diversity at the community level can be interpreted as the diversity of functional strategies of multiple co-occurring species (Villéger et al. 2008, Laliberté and Legendre 2010, Villéger et al. 2013). Finally, phylogenetic diversity quantifies the amount of evolutionary time separating species in the tree of life, often measured as branch length distances among co-occurring species (Webb et al. 2002, Cavender-Bares et al. 2009, Vamosi et al. 2009).

Besides the multidimensional nature of biodiversity (i.e., ranging from genes to species and traits), one can also quantify and interpret biodiversity patterns at multiple spatial scales (Crist et al. 2003, Chao et al. 2012). Taxonomic α-diversity, for instance, refers to the number of species within a given local community, whereas taxonomic γ-diversity is the number of species present across all communities within a region of interest (i.e., regional species diversity; Fig. 1) (Whittaker 1960). Taxonomic β-diversity can be computed as the ratio between α- and γ-diversity and, in this way, it quantifies the differentiation in species identities (or relative abundances) among local communities (Fig. 1) (Tuomisto 2010a, b). Such among community differentiation, however, is not unidimensional as communities can differ in species composition (i.e., some species are replaced by others), species richness (i.e., one community has more species than the other), or both (Fig. 2) (Baselga 2010, 2012).

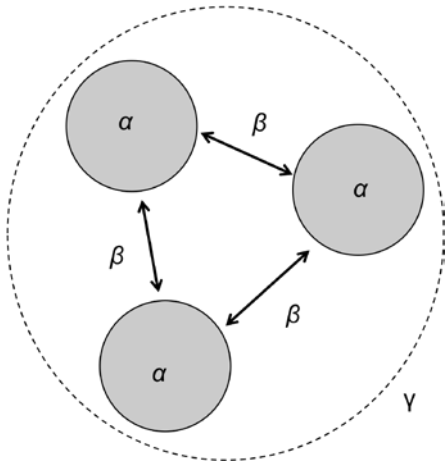

Figure 1. *Conceptual scheme depicting diversity patterns at different spatial scales. α-diversity is the diversity within each habitat patch. B-*

diversity represents differentiation between habitat patches whereas γ-diversity is the total diversity within a region.

A number of *β*-diversity partitioning metrics have been recently developed, allowing one to separate *β*-diversity into its replacement component (i.e., reflecting differences in species composition) and nestedness component (i.e., reflecting richness differences, when the species poor site form a proper subset of the species richer site) (Ulrich et al. 2009, Legendre 2014, Baselga and Leprieur 2015). An advantage of partitioning *β*-diversity into replacement and nestedness components is that it allows a more direct link between processes and biodiversity patterns across spatial scales (Hortal et al. 2011, Leprieur et al. 2011, Angeler 2013, Henriques-Silva et al. 2013). For instance, Fernandes et al. (2013) studied freshwater metacommunity assembly through time in floodplains and found that reduced habitat connectivity during the dry phase resulted in a predominance of metacommunity nestedness. During the wet season, by contrast, habitat patches became much more connected, which resulted in a predominance of replacement due to effective species sorting (Fernandes et al. 2013). This temporal shift from nestedness to replacement, presumably resulting from changes in habitat connectivity, would never have been detected if the analyses had focused only on *β*-diversity. In another example, Henriques-Silva et al. (2013) studied several metacommunities with varying degrees of connectivity and environmental "harshness" and similarly found that nestedness predominates in more isolated and environmentally harsher metacommunities whereas replacement prevails in more connected

and environmentally benign metacommunities. Those observational studies have certainly provided interesting insights about the potential drivers of nestedness and replacement in metacommunities. It is important to realize, however, that observational studies often use spatial distance as a proxy of dispersal rates rather than linking actual dispersal to observed metacommunity patterns. Unfortunately, this approach has intrinsic limitations because the pure spatial signal on nestedness and replacement in metacommunities can result either from dispersal limitation *per se* or from unmeasured environmental conditions that are spatially structured (Peres-Neto et al. 2006). More studies are needed to test whether and how varying dispersal rates interact with environmental conditions to shape nestedness and replacement in metacommunities. Experimental metacommuniy approaches that manipulate dispersal and environmental conditions independently from each other represent an ideal setting to test those ideas in more detail (Logue et al. 2011, Verreydt et al. 2012; see also Chapter I).

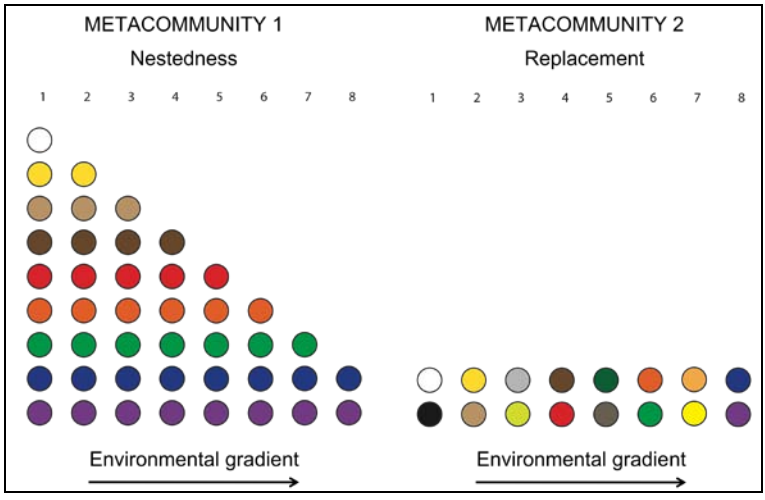

Figure 2. *Conceptual scheme illustrating idealized patterns of nestedness and replacement in metacommunities. Each metacommunity is composed of eight local communities and each coloured circle represents a different species. In metacommunity 1, species poor sites form proper subsets of species richer sites, so that β-diversity in this case derives only from nestedness. Conversely, in metacommunity 2 different species replace each other from site to site along the environmental gradient, so that β-diversity is derived only from replacement. Note that in "real situations" β-diversity is more likely to be determined by both nestedness and replacement, but with different contributions of each component depending on the metacommunity assembly processes (e.g., dispersal, environmental heterogeneity).*

In addition to taxonomy-based patterns of diversity, one can also quantify functional and phylogenetic diversity across spatial scales to attain a more complete picture of community assembly processes (Swenson et al. 2012, Liu et al. 2016). For instance, functional α-diversity is defined as the diversity of functional traits as measured for all species within a local community (Swenson et al. 2012, Swenson 2014). Some indices of functional α-diversity allow detecting patterns of functional clustering versus overdispersion, by comparing observed patterns of trait variance within communities with patterns generated by a null model (Webb et al. 2002, Swenson 2014). In such cases, functional clustering refers to a situation in which species co-occuring in a given community are more similar in trait values to each other than expected by chance (Fig. 3) (i.e., lower trait variance compared to

a null model based on the entire regional species pool). Conversely, functional overdispersion is a pattern in which species are more dissimilar in trait values than expected by chance (i.e., high trait variance within communities; Fig. 3). In analogy to taxonomic β-diversity, functional (trait) β-diversity quantifies the variation in trait composition among local communities (Swenson et al. 2012, Weinstein et al. 2014). It is an interesting task to compare patterns of functional α- and β-diversity (i.e., trait variance and trait composition, respectively) because two communities with the same level of functional α-diversity can be composed by species with very disparate trait values. This is illustrated in Figure 3, in which case there is a strong shift in trait composition between communities A and C, but both are characterized by functional clustering (i.e., a similar pattern of low trait variance within each community).

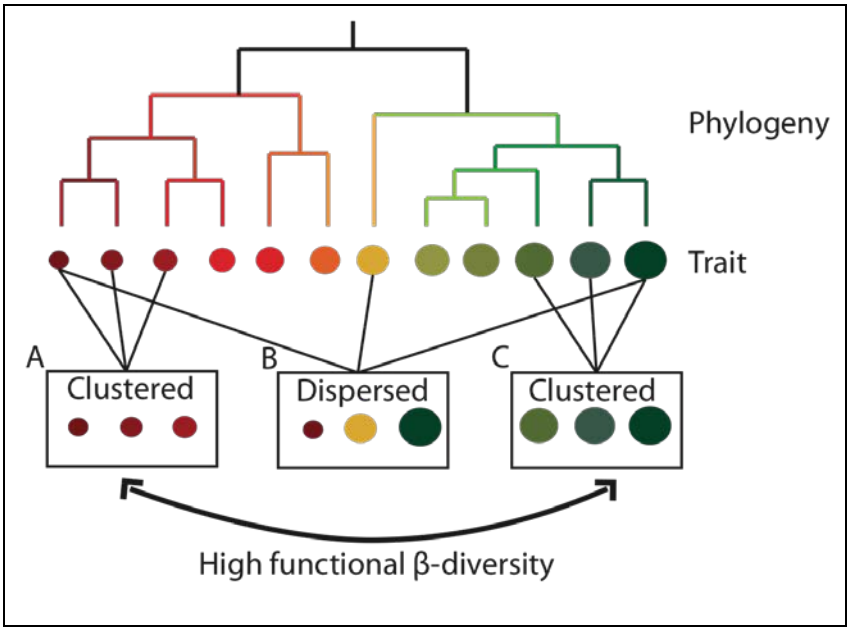

Figure 3. *Conceptual scheme depicts patterns of functional and phylogenetic diversity within and between communities (i.e., functional/phylogenetic α- and β-diversity, respectively). Three local communities are illustrated (A;B;C). Communities A and C have a very similar pattern for α-diversity as they are both composed of only closely related species with very similar trait values (functional and phylogenetic clustering). However, there is high β-diversity between communities A and C as they differ considerably from each other in terms of species trait and phylogenetic composition. Conversely, community B has a pattern of overdispersion (i.e., high α-diversity; high trait variance) but only moderate levels of β-diversity in relation to the other two communities. This illustrates the importance of simultaneously quantifying α- and β-diversity to obtain a more complete picture of metacommunity assembly patterns.*

Phylogenetic α-diversity refers to the amount of evolutionary distinctiveness among species present in a given community (Webb et al. 2002, Webb et al. 2008, Faith 2015). Currently, there are numerous phylogenetic α-diversity indices in the literature and some of those allow detecting patterns of phylogenetic clustering versus overdispersion in a similar way as explained above for functional α-diversity (i.e., comparing observed patterns to null models based on the regional species pool) (Swenson 2014). In analogy to patterns of functional trait diversity, phylogenetic clustering is a pattern in which species within a local community are more closely related to each other than expected by chance, whereas phylogenetically overdispersed communities are characterized by species that are less

related to each other than expected by chance (Fig. 3). Phylogenetic β-diversity quantifies the variation in phylogenetic composition among local communities and helps to shed light on long-term evolutionary processes that shape current species distributions along environmental and spatial gradients (Graham and Fine 2008, Swenson et al. 2012, Duarte et al. 2014). In analogy to patterns of functional trait diversity, it is interesting to compare patterns of phylogenetic α- and β-diversity because two communities with the same phylogenetic structure (e.g., both exhibiting a pattern of phylogenetic clustering) may be composed by completely distinct evolutionary lineages (Fig. 3).

There are considerable advantages of applying a multidimensional and multi-spatial scale approach in studies of community ecology (Weinstein et al. 2014, Liu et al. 2016). One of the most important advantages is that taxonomic, functional trait and phylogenetic approaches can provide complementary insights on the drivers of community assembly (Cavender-Bares et al. 2004, Knapp et al. 2008). For instance, several studies have demonstrated that only a small reduction in species diversity can result in a disproportionately high reduction in functional and phylogenetic diversity after anthropogenic pressures (D'agata et al. 2014, Frishkoff et al. 2014). This happens, for instance, when species with unique trait combinations are more vulnerable to extinction (Young et al. 2016). On the other hand, under a scenario of high functional redundancy within local communities, loss of species diversity will have negligible effects on functional diversity (i.e., if species with redundant traits are lost) (Hillebrand and Matthiessen 2009).

When different species with similar trait values replace each other from site to site along environmental or spatial gradients, functional redundancy emerges at the metacommunity scale (Guelzow et al. 2016). This results in higher taxonomic β-diversity than functional β-diversity, with important consequences for the maintenance of ecosystem processes along environmental or spatial gradients (Rosenfeld 2002, Villéger et al. 2013). Additionally, if measured functional traits are evolutionarily labile functional and phylogenetic patterns can be decoupled (Knapp et al. 2008, Gerhold et al. 2015). For example, a stronger pattern of functional β-diversity than phylogenetic β-diversity would indicate that the measured traits strongly respond to environmental gradients whereas phylogeny would not represent variation in functional traits that respond to the sampled environmental factors (Weinstein et al. 2014). Conversely, a stronger phylogenetic β-diversity than functional β-diversity would indicate that the measured traits are unresponsive whereas phylogenetic distances capture information on unmeasured traits that strongly respond to the sampled environmental factors. In summary, using a multidimensional and multi-spatial scale approach to study community assembly is not trivial and can contribute towards a more predictive science by unrevealing the causes and consequences of different facets of biodiversity.

2. Local and regional drivers of biodiversity

A central goal in community ecology is to understand the mechanisms underlying biodiversity patterns and species distributions

across spatial and temporal scales. Historically, researchers have focused on the importance of species interactions and abiotic conditions within local scales as drivers of species coexistences. The first attempts to incorporate spatial dynamics in biodiversity theory date back to the 1960s with the development of “The Theory of Island Biogeography” (MacArthur and Wilson 1967) and also with the development of the “metapopulation theory” (Levins 1969). The extension of the metapopulation theory to ecological communities (i.e., metacommunity ecology) started only in the 1990s, but its absorption by the scientific community was initially slow (Wilson 1992). It was only after a review paper by Leibold and collaborators (2004) that the metacommunity theory became widespread and widely tested. The consolidation of the metacommunity concept during the last decade increased awareness about the linkage of multiple processes across spatial scales to generate and maintain biodiversity (Leibold et al. 2004, Holyoak et al. 2005, Logue et al. 2011). Such hierarchical, multi-spatial scale assembly processes include abiotic habitat filtering and species interactions occurring at the local scales as well as dispersal from nearby habitats within a regional scale (Shurin 2001, Cottenie et al. 2003, Leibold et al. 2004). Consequently, the metacommunity concept contributed to enrich our understanding of the drivers of biodiversity across spatial scales.

Four metacommunity models that are not mutually exclusive were originally proposed. First, “patch dynamics” is a model that assumes that habitat patches are identical in environmental quality and that a trade-off between species colonization ability and dispersal capacity promotes high diversity at the regional scale (Leibold et al. 2004). It is

predicted that highly connected habitat patches will be dominated by good competitors but poor dispersers, whereas good dispersers but poor competitors will prevail in more isolated habitat patches (Driscoll and Lindenmayer 2009). The “mass-effect” model predicts that extremely high dispersal rates will allow species to occur beyond their niche optima, leading to the homogenization of metacommunities through a mechanism of source-sink dynamics (Mouquet and Loreau 2003). The “neutral model”, in turn, assumes that all species are equal in competitive ability but stochastic processes resulting in local extinction and colonization create spatial variation in species distributions (Rosindell et al. 2011). Finally, the “species-sorting” model assumes that dispersal is not a limiting factor and that local environmental conditions and species interactions are the most important regulators of diversity patterns and species distributions (Leibold et al. 2004).

Some recent reviews and meta-analysis of empirical metacommunity studies revealed that species sorting is often the dominant mechanism shaping species distributions in nature, but pure spatial processes can still contribute significantly to explain variation in species composition among habitat patches (Cottenie 2005, Logue et al. 2011, Soininen 2014, 2016). It is also increasingly recognized that the four idealized metacommunity models often interact to determine species distributions and that their relative contributions vary depending on the spatial and temporal scales considered as well as on the dispersal mode and capacity of the organism groups under investigation (Logue et al. 2011). For instance, Declerck et al. (2011b) have shown that species sorting is the dominant process structuring

Andean cladoceran communities at small spatial scales (i.e., within wetlands), whereas the relative importance of neutral processes increases with increasing spatial scale (e.g., among wetlands), potentially reflecting dispersal limitation at larger spatial scales. In another example, De Bie et al. (2012) have show that the relative importance of spatial processes in structuring aquatic metacommunities increases with body size for passive dispersers whereas species sorting prevails for small organisms (e.g., bacteria and phytoplankton). This indicates that there is a negative correlation between body size and dispersal capacity, especially for passive dispersing organisms.

Another important consideration is that metacommunity processes and patterns are no static, but rather highly dynamic through time. For instance, aquatic metacommunities in flooding plains tend to be structured mainly by dispersal limitation during the dry season (Fernandes et al. 2013). Conversely, in the wet season such metacommunities become much more connected and species sorting prevails (Fernandes et al. 2013). Furthermore, the relative influence of environment and space on metacommunity patterns depends on patch occupancy patterns (Buschke et al. 2015). Specifically, the predictive power of environmental features to explain species distributions tends to be higher for more widespread species than for narrowly distributed ones (Buschke et al. 2015). This implies that the interpretation of metacommunity assembly processes also depends on regional species pool size, sampling grain and extent (Lessard et al. 2011, Chase et al. 2013). A more straightforward interpretation of the role of dispersal and environment in shaping metacommunity structure may be

achieved via metacommunity experiments, in which dispersal and environmental conditions are manipulated (Shurin 2001, Howeth and Leibold 2010, Verreydt et al. 2012, Altermatt et al. 2015), although this approach often lacks the realism and complexity of "natural" metacommunities. Finally, it is noteworthy that the four idealized metacommunity paradigms do not represent the full spectrum of possibilities for metacommunity assembly. This was clearly illustrated by the conceptual scheme presented by Logue et al. (2011), in which the four paradigms were placed in a three-dimensional space defined by the combination of environmental heterogeneity, dispersal and ecological equivalence (Fig. 4). From figure 4, it is clear that the four originally proposed metacommunity models occupy only a small fraction of the theoretical possibilities. On top of that, it is also likely that other axes not represented in figure 4 will also play a role in structuring metacommunities. One such additional axis is metacommunity age or assembly history. For instance, priority effects can significantly influence metacommunity dynamics in situations where first colonizers strongly influence the trajectory of community assembly (Tucker and Fukami 2014, Vannette and Fukami 2014, Fukami 2015, De Meester et al. 2016).

In summary, as the concept of metacommunity matures, it becomes clearer that the relative importance of local and regional scale processes may vary across metacommunities depending not only on the spatial configuration and environmental heterogeneity of habitat patches but also as a function of species traits, assembly history and many other factors. Therefore, it is time to move beyond the four proposed metacommunity models as if they represented all

the potential drivers of metacommunity (Brown et al. 2016). Instead, using a multi-spatial scale and multidimensional perspective (i.e., taxonomic, functional and phylogenetic dimensions) in metacommunity analysis may provide additional insights into metacommunity assembly by approximating species niches and shedding lights on long-term evolutionary processes that underlie current species distributions.

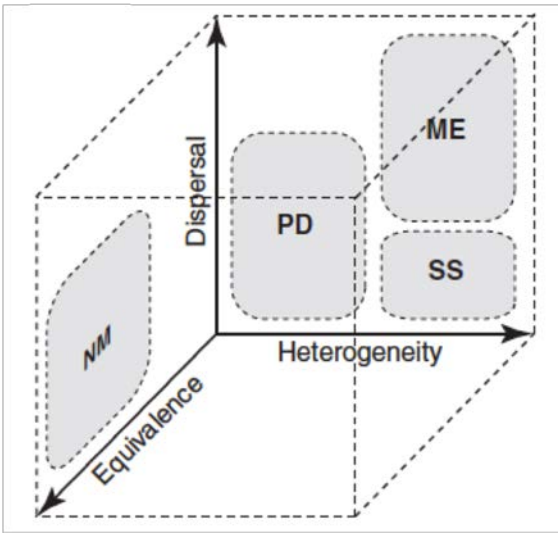

Figure 4. *Position of the four originally proposed metacommunity paradigms along axes of environmental heterogeneity, dispersal and species functional equivalence. Abreviations: NM, neutral model; PD, patch dynamics; ME, mass effects; SS, species sorting (from Logue et al. 2011).*

3. Anthropogenic pressures on biodiversity

In the previous section it was discussed the importance of local and regional scale processes in affecting species distributions in nature. An important topic in current (meta)community studies is how increasing anthropogenic pressures will alter the role local and regional scale processes play on metacommunity patterns. Humans are increasingly modifying natural ecosystems via habitat loss and habitat conversion, species introductions, overexploitation, and changes in biogeochemical cycles to supply an ever-increasing demand for natural resources (Naeem et al. 2012, Pimm et al. 2014). In combination, those different anthropogenic pressures are expected to affect not only environmental quality at the local scale but also the spatial configuration of habitat patches across landscapes (e.g., due to habitat fragmentation).

The current crisis of biodiversity loss can be very pronounced or surprisingly cryptic, depending on the spatial scale considered. At the global scale, anthropogenic-related species extinctions are alarmingly high. Some estimates suggest rates of vertebrate extinctions up to 100 times greater than the historical background (Ceballos et al. 2015), indicating that anthropogenic pressures alone are potentially driving the sixth event of mass extinction in the history of our planet. At somewhat smaller spatial scales, anthropogenic pressures have eradicated many wild populations and resulted in range contractions (Young et al. 2016). This is problematic because many such populations are genetically unique, sometimes showing as great phenotypic differences as that observed between species (Violle et al. 2012, Alberti et al. 2017). Erosion of genetic diversity thus reduces the capacity of species to adapt to global change. In addition, limited

genetic diversity might reduce the fitness of species due to inbreeding (Reed and Frankham 2003). Finally, at the more local spatial scales, environmental changes due to anthropogenic pressures have resulted in loss of individuals within populations or even the complete local extirpation of entire populations (Young et al. 2016). However, such local extinctions are sometimes compensated by the colonization of more common, widespread and generalist species (Monnet et al. 2014). The consequence is that this may result in not net loss of biodiversity locally but in a high temporal turnover as more tolerant species replace vulnerable ones (Dornelas et al. 2014).

The drivers of biodiversity loss similarly range from threats that operate at global scales, such as climate change, to those that act at the landscape scale (e.g., fragmentation), to more local threats, including acute pollution and habitat conversion (Young et al. 2016). The relative importance of each threat varies depending on the region, habitat type and also changes through time. Overall, habitat loss and modification are the main drivers of biodiversity loss in terrestrial and freshwater systems whereas overexploitation predominates in the marine system (overexploitation is also the second main threat for terrestrial systems) (Young et al. 2016). In freshwater systems, the second largest threat for biodiversity is pollution, followed by invasive species (Dudgeon et al. 2005). Finally, climate change is increasingly becoming a global threat across all biomes on earth, but its effects are especially important in alpine systems.

Some recent global analyses have suggested that freshwater species are declining in a much faster rate than marine or terrestrial systems (Young et al. 2016). This disproportionally high risk is not

surprising, as many freshwater ecosystems are embedded in or around centres of high human density (Dudgeon et al. 2005). As such, freshwater fauna is often exposed to multiple stressors, such as heavy metals, pesticides, nutrient inputs and high rates of species invasions (Grimm et al. 2008). In addition, many freshwater systems such as shallow lakes and ponds can be viewed as aquatic islands isolated by a terrestrial matrix, further accentuating their vulnerability to global changes (Hortal et al. 2014).

Indeed, urbanization is an increasingly dominant feature on earth and has been recognized as a major force on biodiversity changes. As human population size increases and cities become denser, several urbanization related selection pressures have been shown to affect species diversity locally and regionally (Urban et al. 2006, Grimm et al. 2008). On the one hand, increasing temperatures (e.g., urban heat-island effect) and pollution in urban areas may filter species based on their niches and influence local species composition (Oke 1973, Arnfield 2003, Kaye et al. 2006, Brans et al. 2016). On the other hand, urbanization may affect the connectivity among habitat patches and thereby influence dispersal rates among populations and communities (Urban et al. 2006). Another important type of anthropogenic pressure is eutrophication, which may result from agriculture, amongst other causes (Ulén et al. 2007, Withers and Haygarth 2007). Eutrophication has been shown to affect species diversity of a large number of organism groups both in terrestrial and aquatic ecosystems (Declerck et al. 2005, Kruk et al. 2009, Hautier et al. 2015). Therefore, ecologists increasingly face the challenge of not only understanding biodiversity patterns in nature, but also of predicting how increasing

anthropogenic pressures will affect biodiversity patterns and associated ecosystem processes across spatial scales.

4. Biodiversity – ecosystem functioning relationship

Hundreds of experiments have been conducted over the last two decades to investigate the effects of species diversity on ecosystem processes (Cardinale et al. 2006). There is now a general consensus that increasing biodiversity (e.g., number of species) enhances a variety of ecosystem functions, such as decomposition rates, nutrient cycling, primary production and the control of algal blooms in lakes (Cardinale et al. 2012, Lefcheck et al. 2015). It is also well recognized that such positive relationship between biodiversity and ecosystem functioning is non-linear and saturating (Fig. 5) (Cardinale et al. 2012). This means that as more and more species get extinct from ecosystems, the reduction in ecosystem processes accelerates disproportionally fast. Additionally, the positive effect of biodiversity on ecosystem functions becomes more evident in studies that measure different types of ecosystem processes, so that biodiversity effects are stronger when considering the multi-functional aspects of ecosystems (Maestre et al. 2012, Soliveres et al. 2016). Finally, there is a current consensus that two main types of ecological processes mediate the relationship between biodiversity and ecosystem functioning [but see van der Plas et al. (2016) for a third proposed mechanism coined "Jack of All Trades"]. First, more diverse ecological communities tend to exhibit higher species complementarity via niche partitioning, so that increasing diversity results in a more effective depletion of

resources and transfer of organic matter and energy to higher trophic levels (Loreau and Hector 2001, Kahmen et al. 2006). Secondly, more diverse ecological communities have a higher probability of hosting a dominant species, which is very efficient in up-taking resources and transferring it to higher trophic levels (this second mechanism is called selection effect) (Loreau and Hector 2001, Carroll et al. 2011). Interestingly, approximately half of the studies reviewed by Cardinale et al. (2012) have provided evidence for complementarity and the other half for selection effect as the main mechanism mediating ecosystem processes. Yet, there is some evidence that there is a shift from selection effects to complementarity as the main mechanism underlying ecosystem processes as experiments run longer (Cardinale et al. 2007). This shift is likely to happen because species that dominate a given community at a certain moment may be replaced by other species as environmental conditions fluctuate through time, which leads to increasing complementarity on the long-term and this also enhances the stability of ecosystem functions through time (Downing et al. 2014).

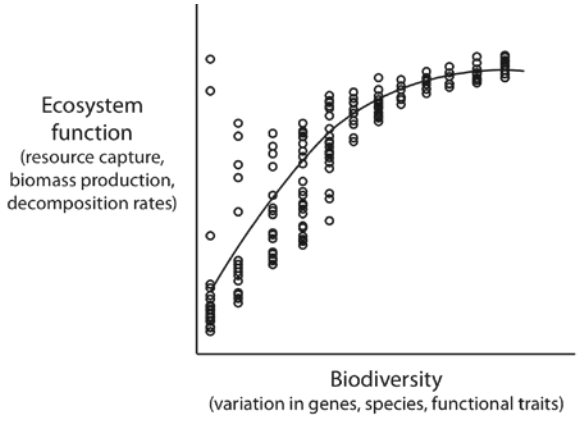

Figure 5. *The form of a typical biodiversity-function relationship. This conceptual diagram summarizes what we know about the shape of the biodiversity-ecosystem functioning relationship based on hundreds of experiments (reviewed by Cardinale et al. 2012). The line represents the average change of ecosystem functions with increasing levels of biodiversity at the genetic level, species level or functional trait level. Note that uncertainty is higher for species poor communities because monocultures can either perform a single function at the highest or lowest level, depending on species traits. Also, the relationship is non-linear and saturating (adapted from Cardinale et al. 2012).*

Despite all this cumulative knowledge on the relationship between biodiversity and ecosystem functioning over the last two decades, most studies manipulating the number of species within communities have reported high levels of variation in ecosystem processes for the same number of species (Cardinale et al. 2006). Considering the high levels of biodiversity loss and the need to prioritize conservation efforts, an important next step for ecologists is to provide more accurate predictions of the biodiversity – ecosystem functioning relationship. There is evidence that directly accounting for functional trait diversity or phylogenetic diversity instead of only taxonomic diversity results in more accurate predictions of ecosystem functioning by approximating species niches (Cadotte et al. 2009, Flynn et al. 2011, Cadotte et al. 2012, Srivastava et al. 2012b, Cadotte 2013, Thompson et al. 2015).

5. Trait-based approaches in ecology

Functional traits are defined as any morphological, physiological, behavioural or phenological characteristics of an organism affecting its individual performance (Hillebrand and Matthiessen 2009). Functional traits largely determine species interactions and abiotic tolerances along ecological gradients. Therefore, trait-based metacommunity analyses may provide more accurate predictions of how species interact and respond to changes in environmental conditions (McGill et al. 2006, Violle et al. 2012). Moreover, a number of functional traits of species directly translate into ecosystem functions (e.g. metabolic rates in bacteria, photosynthetic rates in algae and plants, grazing rates in herbivores) (Hillebrand and Matthiessen 2009, Guelzow et al. 2016). Trait-based metacommunity ecology may thus help understanding how community characteristics and associated ecosystem functions vary along environmental gradients with increasing anthropogenic pressures. Under a scenario of global change, for instance due to increasing urbanization and agricultural land use, one can expect higher temperatures, pollution, nutrient inputs and isolation in freshwater ecosystems, with serious consequences for biological conservation and the maintenance of ecosystem functions (Robin et al. 2013, Symons and Arnott 2013, Allan et al. 2015, Brans et al. 2016). Trait-based metacommunity ecology does not only contribute to conservation issues, such as ecosystem management and environmental policy, but also to the development of a sustainable economy and society, by linking local

and regional patterns in species composition and diversity to ecosystem services.

An important task in modern community ecology is to understand whether and how global change affects functional trait diversity within and among communities and also to elucidate which species characteristics make them more vulnerable to environmental change. In this context, traits can be classified in terms of their response to environmental changes (response traits) and their impacts on ecosystem processes (effect traits) (Suding et al. 2008). Several recent studies on "community disassembly" have show that species extinctions after disturbance are not a random process but rather depend on species response traits (Larsen et al. 2005). The more obvious consequence of such non-randon species losses is that it disproportionally impacts distinct regions of the functional trait space (and often distinct regions of the evolutionary tree of life as well; Fig. 6) (Faith 2015). Furthermore, depending on the correlation between response and effect traits, such non-random species extinctions can trigger an early collapse of ecosystem functions (Díaz et al. 2013, Oliver et al. 2015). Specifically, a positive correlation between response and effect traits indicates that the more vulnerable species to a given disturbance are also the ones that contribute the most to ecosystem processes (Larsen et al. 2005). Such type of functional relationship between response and effect traits is expected to reduce the resistance of ecosystems to disturbance, resulting in a shift to an alternative stable state (Oliver et al. 2015). This may be the case in aquatic systems subject to increased urbanization pressure, as large zooplankton species may be more vulnerable to urbanization (Brans et

al. 2016), but they are also considered superior grazers (Mourelatos and Lacroix 1990, Gianuca et al. 2016b) (see also Chapter IV). The elimination of larger zooplankton in urban ponds may therefore trigger a state shift from a clear-water system to a turbid state, although other factors can synergistically influence this transition, remarkably the presence of aquatic vegetation tends to stabilize clear-water ponds (Scheffer 2004). Conversely, a negative relationship between response and effect traits implies that the more vulnerable species to extinction actually contribute little to ecosystem processes (Larsen et al. 2005). The predicted outcome of such negative relationship is that ecosystems will be more resistant to disturbance, because highly tolerant species will still maintain elevated levels of ecosystem processes, whereas the extinction of several functionally irrelevant species will have only a negligible effect on the maintenance of ecosystem functions (Larsen et al. 2005, Oliver et al. 2015).

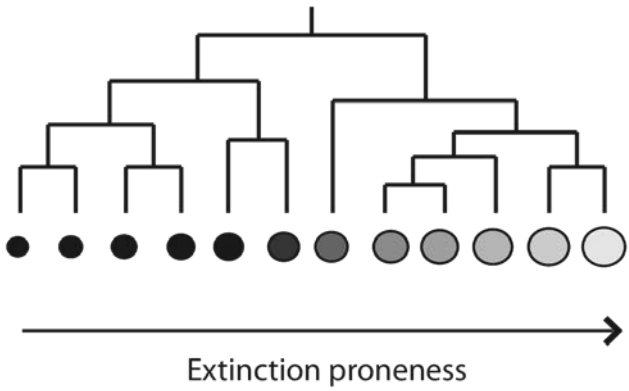

Figure 6. *Scheme demonstrating how species response traits may affect community trait and phylogenetic structure. In this example,*

larger species (circles) are more prone to extinction (light colours) after disturbance (e.g., heat waves, pollution, introduction of size-selective predators). As a result, the community will tend to be dominated by small, closely related species after disturbance (black colours).

In addition to the classification of traits based on their responses to disturbance and their effects on ecosystems, one can also classify traits in terms of their position along axes of interaction strengths, specifically fitness (competitive ability) and niche differences (Mayfield and Levine 2010, HilleRisLambers et al. 2012). In cladoceran zooplankton, for instance, body size has been repeatedly demonstrated to determine differences in competitive ability (i.e., it determines fitness differences *sensu* Mayfield and Levine 2010). The zooplankton size-efficiency hypothesis postulates that larger species are more efficient in filtering particulate matter from the water column and also have a more efficient conversion of food in egg production (Brooks and Dodson 1965, Dodson 1974). However, larger zooplankton species are also more vulnerable to size-selective fish predation pressure (Brooks and Dodson 1965). Consequently, it is common to observe a strong shift from large-dominated zooplankton communities in the absence of fish to small-dominated communities in systems with high predation pressure (Brooks and Dodson 1965). In addition, larger zooplankton species show reduced fitness under higher temperatures and pollutants due to physiological constraints (Moore and Folt 1993).

It is important to realize that, besides the importance of body size, other less explored zooplankton functional traits might be very relevant in determining species coexistences (Barnett et al. 2007, Vogt et al. 2013). For instance, a strong candidate trait that likely determines zooplankton **niche differences** is feeding type (e.g., scrapers versus filter-feeders), as differences in this trait may allow species with very disparate body size and competitive abilities to coexist (Levine and HilleRisLambers 2009). Another example of a potentially relevant zooplankton trait is the degree in which species are associated with or live attached to plants, as this may determine the level of micro-habitat segregation among pelagic versus plant associated species in ponds (Barnett et al. 2007, Vogt et al. 2013). The latter trait may therefore allow species with disparate competitive abilities to coexist via micro-habitat segregation.

Another increasingly relevant topic in functional trait research is to understand how contrasting trait responses along environmental and spatial gradients affect our capacity to interpret community assembly patterns. For instance, in a recent study, Butterfield et al. (2013) demonstrated that some traits increase in variance along a moisture gradient whereas other traits decrease in variance. Such opposed trait responses along the moisture gradient cancelled out each other's signal, so that mixing up such contrasting traits into a single trait metric resulted in a non-significant shift in trait variance along the gradient (Butterfield et al. 2013). This observation is against the general intuitive idea that the more traits one includes in functional indices, the more informative they are. In addition, some traits may be more influenced by regional scale assembly processes whereas other

traits may be more responsive locally and we do not fully understand how trait-spatial scale mismatches affect our capacity to interpret community assembly patterns. For instance, species coexistence within communities is mainly determined by traits involved in resource exploitation, microhabitat use, or diet (Ackerly et al. 2006). Silvertown et al. (2006) used the term α-niche to refer to this local scale dimension of niche differentiation. Conversely, at larger spatial scales species are sorted based on their habitat preferences or environmental tolerances (Cavender-Bares et al. 2006, Cavender-Bares et al. 2009). The term β-niche (Silvertown et al. 2006) has been used to refer to this larger-scale component of niche differentiation as it defines the positioning of species along environmental gradients. An intuitive hypothesis is that α- and β-niche traits will have very different responses to processes operating at different spatial scales and that combining such traits into single metrics may introduce confounding effects in the analysis due to trait-spatial scale mismatches.

6. Phylogenetic-based approaches in ecology

Despite the advantages of using trait-based approaches in ecology, as highlighted in the previous section, a potential weakness of this approach is that the power of trait-based analyses relies on the relevance of the measured traits for ecosystem functioning and for species responses to environmental conditions (Flynn et al. 2011). In practice, however, choosing which traits are to be measured is a decision made a priory by investigators and the measured traits may

not always represent the multidimensional niches of species (Cadotte et al. 2008). Besides that, measuring all the relevant traits for multiple species is often unfeasible for many organism groups. An alternative approach is using phylogenetic distances as a proxy of niche differentiation among species (Webb et al. 2002, Vamosi et al. 2009). Phylogenetic distances may be used as a representation of species similarities and differences under the assumption that functional traits are conserved along the phylogeny (Fig. 7a) (Wiens et al. 2010, Mouquet et al. 2012). Indeed, there is mounting evidence that distance in evolutionary time separating species in the tree of life tends to correlate with diversification in functional traits (Blomberg et al. 2003). Many recent studies have demonstrated that phylogenetic-based approaches synthetically represent species multidimensional niches and, therefore, can provide more accurate predictions of the drivers of (meta)community assembly than analyses based on a few measured traits or taxomony alone (Leibold et al. 2010, Baraloto et al. 2012). There is also increasing evidence that phylogeny-based approaches provide more accurate predictions of the relationship between biodiversity and ecosystem functioning (Flynn et al. 2011, Cadotte 2013, Thompson et al. 2015). However, it is noteworthy that when functional traits are labile (Fig. 7b) (i.e., not conserved along the phylogeny) using phylogenetic distances may be misleading in ecological analyses (Cavender-Bares et al. 2004, Gerhold et al. 2015).

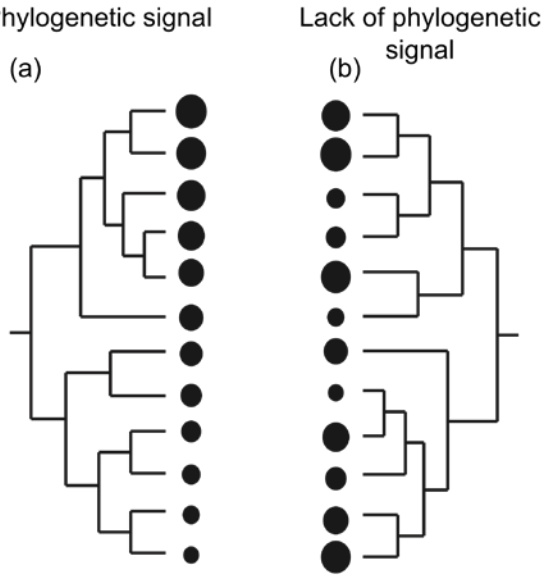

Figure 7. *Scheme representing (a) phylogenetic signal in the measured trait (circle size), indicating that more closely related species are more similar to each other in size; and (b) lack of phylogenetic signal, showing that more closely related species do not always resemble each other in size (i.e., because size varies randomly along the phylogeny).*

7. Integrating trait and phylogenetic distances

In the last two sections above (i.e., sections 5 and 6), the advantages and potential pitfalls of using either trait- or phylogenetic-based approaches in analyses of metacommunity assembly and ecosystem functioning were discussed. Here, we advocate that an important next step in metacommunity analysis and ecosystem functioning research is to integrate both, trait and phylogenetic information into a single framework. This can be accomplished via a

trait-phylogenetic weighing parameter, which allows investigators to assess the relative contribution of traits and phylogeny to observed ecological patterns (Cadotte et al. 2013, Gianuca et al. 2016a). A clear advantage of integrated approaches is that the two pieces of information (trait and phylogeny) can be decoupled and that they can be highly complementary in explaining community assembly patterns. In such cases, using trait or phylogenetic data alone will reveal only part of the whole ecological story and thus result in reduced explanatory power in analysis of community assembly and ecosystem functioning. For instance, Cadotte et al. (2013) demonstrated that measured traits can be labile and relevant for community assembly while phylogeny can account for information in other unmeasured traits that are also relevant, so that combining trait and phylogenetic distances enhanced the power of the analysis because they provided complementary information on different assembly mechanisms. Another key advantage of integrating trait and phylogenetic data is that one can map patterns of trait evolution directly on the phylogeny (e.g., using an evolutionary trait-gram). In this way, it is possible to understand how long term evolutionary processes, such as those leading to trait convergences and divergences, affect current species interactions and distributions along environmental and spatial gradients.

8. Ponds and shallow lakes as model systems in ecology

Ponds and shallow lakes are considered ideal model systems to study metacommunity assembly and dynamics because they represent

well-defined entities isolated from each other through a matrix of terrestrial ecosystems (De Meester et al. 2005, Hortal et al. 2014). Moreover, these systems normally vary considerably in environmental conditions along gradients of land-use (Declerck et al. 2006), which tends to strongly influence metacommunity organization (Logue et al. 2011). Despite the long-standing contribution of freshwater ecosystems for ecological theory as a whole, and specially the relevance of zooplankton communities of ponds and shallow lakes for metacommunity ecology [e.g., (Shurin 2001, Cottenie et al. 2003, Cottenie and De Meester 2004, Urban 2004, Declerck et al. 2011b)], trait- and phylogenetic approaches are relatively underappreciated in these systems compared to terrestrial ones. In this PhD research we demonstrate that ponds and shallow lakes represent a fertile ground to uncover mechanisms of metacommunity assembly and ecosystem functioning via integrated analysis of trait and phylogenetic data.

9. Thesis outline

This PhD thesis is developed around three main research questions. First, what is the relative importance of local environmental quality, dispersal, and regional environmental heterogeneity on biodiversity patterns across spatial scales? Secondly, how anthropogenic pressures associated with urbanization and land-use intensity influence the functional and phylogenetic diversity of zooplankton metacommunities? Thirdly, what is the relative predictive power of functional and phylogenetic diversity of zooplankton communities on a key ecosystem function, top-down control of algae?

To tackle the abovementioned research questions we used a combination of experimental and fieldwork approaches and applied taxonomic, functional trait and phylogenetic statistical methods. Although each approach and technique applied has a different focus, they clearly provide complementary perspectives on the research questions investigated. On the one hand, the experimental approaches provide a proof of principle on the potential mechanisms shaping metacommunity assembly and ecosystem functioning, but they represent only a simplified picture of the natural world. On the other hand, field observations lack the power of replication and manipulation but allow validation of experimental results to more complex and natural ecosystems.

This PhD thesis consists of four chapters. In **chapter I** we used data from a factorial metacommunity experiment to test how dispersal and environmental heterogeneity affect different components of β-diversity, namely replacement and nestedness. Recent observation studies on this topic suggest that spatial isolation mainly affects β-diversity via nestedness whereas increased connectivity affects β-diversity via species replacement (Fernandes et al. 2013, Henriques-Silva et al. 2013, Bender et al. 2016). Whereas this is a logic situation, a potential limitation of observational studies is that they use spatial distance as a proxy of dispersal rather than linking actual dispersal rates to diversity patterns. Unfortunately, this has intrinsic limitations because the pure signal of spatial isolation can result either from dispersal limitation per se or from unmeasured environmental variables that are spatially structured (Peres-Neto et al. 2006). In addition, it is not clear from observational studies whether the

association between spatial isolation and nestedness is dependent on the interaction between environmental harshness and dispersal limitation. To overcome those limitations we revisited an experimental dataset in which dispersal rates and nutrient conditions were manipulated independently (Fig. 8) (Verreydt et al. 2012). We hypothesized that increasing dispersal would reduce nestedness and enhance the relative contribution of replacement to β-diversity in heterogeneous landscapes, as previously suggested (Fernandes et al. 2013, Henriques-Silva et al. 2013, Bender et al. 2016). However, we also expect distinct patterns of nestedness and replacement in environmentally homogeneous landscapes. Specifically, we hypothesize that lack of dispersal will result in high species replacement due to stochastic drift in homogeneous landscapes.

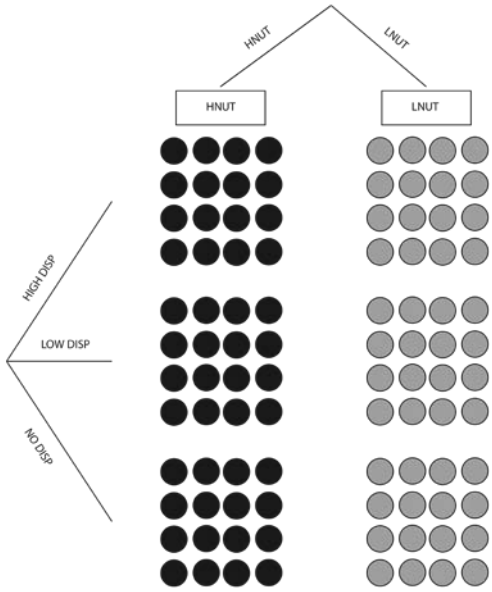

Figure 8. *Scheme depicts the experimental design in which three dispersal levels and two nutrient levels were manipulated. Each*

factorial combination of nutrient and dispersal was composed of 16 mesocosms. Each of those 16 mesocosms was inoculated with a zooplankton community from a single lake, so that communities of 16 different lakes were present when considering all 16 mesocosms combined (regional pool). Note that each of the 16 zooplankton sources was used to inoculate 6 different mesocosms (i.e., each mesocosm representing one of the six possible combinations of nutrient and dispersal). In the high dispersal treatment, however, each mesocosm started with a zooplankton community from a single lake (80%) plus a mixture of all 16 lakes combined (20%). This is just a schematic visualization and mesocosms were randomized in space. Abbreviations: HNUT = high nutrients; LNUT = low nutrients; HIGH DISP = high dispersal; LOW DISP = low dispersal; NO DISP = no dispersal (for details see Methods section in Chapter I).

In **chapter II** we used a hierarchical sampling design and applied a multidimensional approach, including taxonomic, functional trait and phylogenetic data, to investigate how urbanization may be affecting different facets of biodiversity across several spatial scales. We hypothesize that zooplankton species responses to urbanization would largely depend on species traits and evolutionary relatedness. If this is the case, we expect that using trait- and phylogeny-based approaches would enhance the explained variance of metacommunity drivers compared to more traditional, taxonomy-based approaches that treat species as equally differentiated. In **chapter III** we developed a conceptual framework for integrating trait and phylogenetic distances in multi-spatial scale community analysis. We hypothesize that

different traits would respond to processes at different spatial scales, so that mixing such traits into a single diversity metric would compromise the power of the analysis. We demonstrated that our approach, which uses phylogenetic distances as a benchmark to scale the explanatory power of different traits across spatial scales, is key to enhance the power and interpretability of community analysis. Finally, in **chapter IV** we used a creative experimental design to disentangle the role of functional trait (body size) and phylogenetic diversity on a key ecosystem function, namely zooplankton top-down control of algae. Our design allowed us to test three main hypotheses: (i) phylogenetic distances better approximate species similarities and differences than a single trait (body size) and therefore are more informative to predict top-down control than body size alone; (ii) phylogenetic distances complement the information provided by body size, so that considering the two pieces of information improve predictions of top-down control; and (iii) phylogenetic distances do not capture any additional ecological information relevant to predict top-down control whereas body size is a key trait that is highly informative to predict top-down control. The overarching goals of the thesis were to quantify the relative importance of local and regional processes to structure different dimensions of diversity (i.e., taxonomic, functional and phylogenetic) and to assess the power and complementarity of different diversity dimensions to predict ecosystem function.

CHAPTER I

EFFECTS OF DISPERSAL AND ENVIRONMENTAL HETEROGENEITY ON THE REPLACEMENT AND NESTEDNESS COMPONENTS OF *B*-DIVERSITY

[Modified from Ecology (2016), doi: 10.1002/ecy.1666]

Andros T. Gianuca, Steven A.J. Declerck, Pieter Lemmens and Luc De Meester

ABSTRACT

Traditionally metacommunity studies have quantified the relative importance of dispersal and environmental processes on observed β-diversity. Separating β-diversity into its replacement and nestedness components and linking such patterns to metacommunity drivers can provide richer insights into biodiversity organization across spatial scales. Yet, it is very difficult to measure actual dispersal rates in the field and to define the boundaries of natural metacommunities. To overcome those limitations, we revisited an experimental metacommunity dataset in which dispersal and nutrient levels were manipulated independently. We tested the independent and interacting effects of landscape-wide environmental heterogeneity and dispersal on each component of β-diversity. We show that the balance between the replacement and nestedness components of β-diversity resulting from eutrophication changes completely depending on dispersal rates. Nutrient enrichment negatively affected local zooplankton diversity and generated a pattern of β-diversity derived from nestedness in unconnected, environmentally heterogeneous landscapes. Increasing dispersal erased the pattern of nestedness, whereas the replacement component gained importance. In environmentally homogeneous metacommunities, dispersal limitation created community dissimilarity via species replacement whereas the nestedness component remained low and unchanged across dispersal levels. Our study clearly illustrates the importance of separating β-diversity into its replacement and nestedness components to fully link processes and patterns in metacommunity analysis.

Introduction

Biodiversity is a multifaceted concept that requires a multiscale approach to be fully understood (Segre et al. 2014). In addition to local scale diversity (α-diversity), differentiation among habitats (β-diversity) is an important determinant of regional diversity. β-diversity, here defined as the dissimilarity in species composition among a pair of sites, can be partitioned into two components: dissimilarity derived from species replacement and dissimilarity derived from nestedness (Baselga 2010, Legendre 2014). The replacement component reflects changes in species identities (or relative abundances) between sites, whereas the nestedness component reflects to what extent the species-poor site contains a proper subset of the species-richer site (Baselga 2012, Legendre 2014). The replacement and nestedness components can contribute jointly to total dissimilarity among communities, but their relative importance will change depending on the ecological processes structuring metacommunities (Brendonck et al. 2015, Tonkin et al. 2016). Therefore, partitioning β-diversity into its replacement and nestedness components and linking such patterns to metacommunity drivers can provide additional insights into the mechanisms that shape biodiversity patterns across spatial scales (Hortal et al. 2011, Leprieur et al. 2011, Ewers et al. 2013). Depending on the relative contribution of each component to total β-diversity, different conservation strategies are needed to preserve regional species diversity (Angeler 2013). For instance, a dominance of the nestedness component of dissimilarity means low complementarity among sites and implies the need of prioritizing sites with high α-diversity, while a predominance

of the replacement component would require the conservation of multiple sites at the landscape scale. In order to protect regional biodiversity, it is therefore crucial to understand what ecological phenomena shape each component of β-diversity (i.e., nestedness and replacement). The latter allows one to fully link scale dependent processes to biodiversity patterns (Leprieur et al. 2011, Angeler 2013) and hence effectively inform management practices.

The metacommunity approach provides a strong conceptual framework to investigate the extent to which local environmental conditions interact with dispersal in determining biodiversity patterns at local and regional spatial scales (Shurin 2001, Leibold et al. 2004, Cottenie 2005, Logue et al. 2011). Traditionally, however, studies investigating the drivers of replacement and nestedness have focused at a biogeographical scale, and most metacommunity studies have not distinguished the two components of β-diversity [but see (Brendonck et al. 2015, Tonkin et al. 2016) for some recent examples]. At the biogeographical scale (i.e., assuming a history of very low or no dispersal among regions), spatial isolation mainly results in species replacements (i.e., spatial turnover) due to the long-term evolutionary processes of speciation and extinction, which creates differences among regional species pools (McKnight et al. 2007, Melo et al. 2009, Leprieur et al. 2011). Conversely, spatial isolation has been suggested to increase the relative importance of nestedness and reduce the importance of species replacement (i.e., spatial turnover) at the metacommunity scale (Henriques-Silva et al. 2013, Bender et al. 2016). Yet, it is not entirely clear if this relative increase in nestedness is solely driven by spatial isolation or rather depends on the

interaction between isolation and environmental constraints (Henriques-Silva et al. 2013). Although dispersal has been suggested to play a central role in determining β-diversity and its components across spatial scales [e.g., (Hortal et al. 2011, Leprieur et al. 2011, Tonkin et al. 2016)], the majority of observational studies use spatial variables as an indirect proxy of dispersal rates rather than linking the patterns of diversity directly to actual dispersal rates. This has intrinsic limitations, because pure spatial signals on β-diversity patterns in observational studies can result either from dispersal limitation or from unmeasured environmental variables that are spatially structured (Peres-Neto et al. 2006, Dray et al. 2012). While it is often difficult to measure dispersal rates in the field, dispersal can be controlled in experiments. Experiments with metacommunities therefore allow a more direct assessment of the potential role of dispersal in shaping diversity patterns, while additional factors can be controlled for (Logue et al. 2011, Verreydt et al. 2012).

The main goal of this study is to experimentally investigate potential drivers of the nestedness and replacement components of β-diversity in metacommunities. We expect that decreasing dispersal rates, for instance due to increasing fragmentation, will result in an increase of the nestedness component of β-diversity in environmentally heterogeneous landscapes (Fig. 1a) (Henriques-Silva et al. 2013, Bender et al. 2016). In more connected metacommunities the contribution of the nestedness component may be reduced because dispersal rescues species from local extinction (Hanski 1998). At the same time, high dispersal allows species to track suitable environmental conditions at the regional scale (Cottenie and De

Meester 2004, Leibold et al. 2004), increasing species replacement via species sorting. Therefore, we anticipate a decrease in the relative contribution of the nestedness component and an increase in the replacement component to total β-diversity with increasing dispersal rates in environmentally heterogeneous landscapes (Fig. 1a). Only under extremely high dispersal species replacement will decrease due to mass effects (Mouquet and Loreau 2003). Ultimately, such a shift from the nestedness to the replacement component may stabilize total β-diversity along a broad range of dispersal rates and landscape connectivity scenarios (Fig. 1a).

We expect distinct patterns of β-diversity derived from nestedness and replacement along dispersal gradients in environmentally homogeneous landscapes. We anticipate that the nestedness component in homogeneous landscapes will be low and barely change along the dispersal gradient for two reasons. First, although we expect some random species extinctions in homogeneous, unconnected landscapes, nestedness is all about non-random, directional species losses (Legendre 2014). Secondly, increasing dispersal in environmentally homogeneous landscapes can result in rapid homogenization of metacommunities (Declerck et al. 2012), which reduces differences in α-diversity and maintains the nestdeness component of β-diversity low across the dispersal gradient (Fig 1b). In contrast, we expect that in the absence of dispersal, a certain degree of species replacement will be generated by stochastic drift (Fig. 1b) (Logue et al. 2011). Increasing dispersal will lead to convergence in species composition due to homogenization (Fig. 1b) (Mouquet and Loreau 2003).

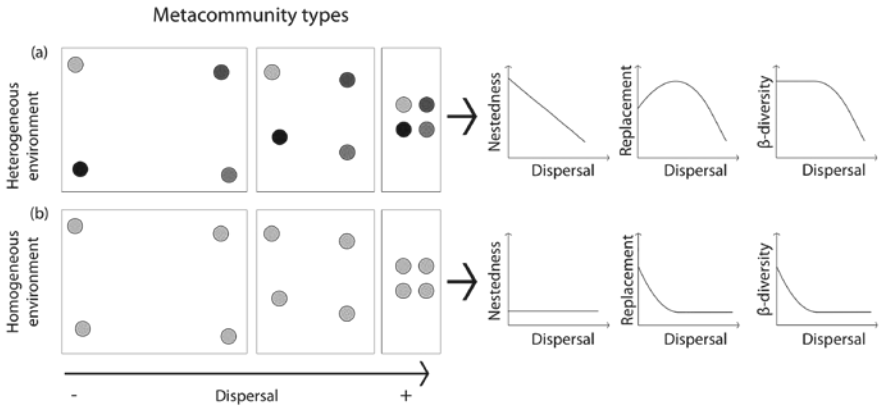

Figure 1. *Conceptual scheme representing the expected relationships between environmental heterogeneity, dispersal rates and community dissimilarity derived from nestedness and replacement in metacommunities. In a landscape characterized by environmental heterogeneity (e.g., represented by different colors in panel a), the nestedness component is expected to decrease with increasing dispersal because species are rescued from extinction, whereas the replacement component is enhanced with increasing dispersal rates up until a point where mass-effects start to homogenise species composition. The result is that β-diversity remains high, but has different proportions of the nestedness versus the turnover components until landscape connectivity is so high that mass effects come into play. In homogeneous landscapes (represented by similar colors of the circles in panel b), the nestdness component may not significantly change along the dispersal gradient (see text for details). Strong dispersal limitation in homogeneous landscapes can cause a pattern of species replacement due to stochastic variation, while increasing dispersal in homogenous environments rapidly reduces replacement due to homogenization. We note that the responses to*

environmental heterogeneity as shown in the figure only apply to gradients of environmental stressors that exclude species and can lead to nestedness (e.g., eutrophication, pollution, acidification).

We here investigate the independent and interacting effects of dispersal and environmental heterogeneity on the relative contribution of the nestedness and replacement components to β-diversity of zooplankton metacommunities. Eutrophication is an important anthropogenic pressure worldwide and has been shown to strongly reduce biodiversity in multiple organism groups in shallow lakes and ponds (Scheffer 2004, Declerck et al. 2005, Kruk et al. 2009). Eutrophication gradients may result in species losses and generate community dissimilarity via nestedness. Alternatively, eutrophication gradients may also drive species replacement if species differ in their tolerances to eutrophication (Declerck et al. 2007). Yet, it is largely unknown whether and how varying dispersal rates would alter the relative importance of the nestedness and replacement components resulting from eutrophication. We hypothesize that the influence of eutrophication on the nestedness and replacement components of β-diversity changes depending on dispersal rates and environmental heterogeneity following the patterns depicted in the conceptual Figure 1. To test this, we reanalysed data from a mesocosm experiment in which nutrient concentrations and dispersal rates were manipulated independently (Verreydt et al. 2012). Specifically, we test three key ideas: (i) eutrophication leads to directional species losses due to differences in susceptibility to eutrophication, which enhances the importance of the nestedness component of β-diversity in less

connected, heterogeneous landscapes; (ii) increasing dispersal enhances the importance of the replacement and reduces the importance of the nestedness component in heterogeneous landscapes; (iii) increasing dispersal reduces the importance of replacement and total β-diversity in homogeneous landscapes, whereas the nestedness component remains low and unchanged across the dispersal levels. We tested our predictions by focusing on both the metacommunity of mesocosms exposed to different nutrient levels (heterogeneous landscape) and exposed to the same nutrient level (homogeneous landscape) across the dispersal levels.

METHODS

Experimental design and sampling

We used data from a cross-factorial pond mesocosm experiment (n=96) (Verreydt et al. 2012) in which three levels of dispersal and two levels of nutrient concentrations were manipulated (see details below). At the start of the experiment (23 May 2006), 96 plastic containers were filled (volume: 200 L) with a mixture of 120 L distilled water and 60 L tap water. The nutrient treatments were established 5 days after filling the mesocosms through addition of phosphate (KH2PO4) and nitrogen (NaNO3). Initial nutrient additions were equivalent to 1000 µg P L^{-1} and 16 000 µg N L^{-1} in the high nutrient (HNUT) mesocosms and 10 µg P L^{-1} and 160 µg N L1 in the low nutrient (LNUT) mesocosms. A tenth of these concentrations were added weekly to maintain nutrient concentrations throughout the experiment. Phytoplankton and bacterioplankton were inoculated just after the nutrient addition took place (i.e., fifth day). After we

observed a consistent difference in phytoplankton biomass between LNUT and HNUT treatments (day 32), we inoculated zooplankton. Zooplankton communities from 16 different shallow lakes in Belgium were used to inoculate blocks of six mesocosms per lake (6 x 16 = 96 mesocosms) [see Verreydt et al. (2012) and supplementary information therein for more details about the lakes and communities].

Within each set of six mesocosms, we created two levels of nutrient addition (low and high) and three levels of dispersal intensity (no dispersal, low dispersal and high dispersal). In each block (i.e., zooplankton source), the no dispersal (NDISP) and low dispersal (LDISP) mesocosms were inoculated with zooplankton originating from one single lake, whereas the high dispersal (HDISP) mesocosms were initially inoculated with 80% of zooplankton from the corresponding single lake and a plankton mixture containing zooplankton from all 16 lakes (i.e. 20%). All dispersal levels started with the same regional species pool (see also Appendix S1: Figure S4), meaning that inocula from all 16 lakes were present when combining all mesocosms of a given dispersal level (n = 32). In contrast to the other two dispersal treatments, however, in the HDISP treatment we created a situation in which all habitat patches were already connected at the start of the experiment, so that each species present in the regional species pool was given a chance to enter each local community from the beginning. The mesocosms of the NDISP treatment were kept isolated from the rest of the experimental metacommunity throughout the entire duration of the experiment. The other two dispersal levels were achieved by manually exchanging water among mesocosms. For this, water was collected from all

mesocosms of the respective dispersal level (n = 32) and the pooled volume was redistributed in equal parts over the same mesocosms again. We exchanged 40 mL per mesocosm for the LDISP whereas the high dispersal level (HDISP) was achieved by exchanging 2 L volumes at weekly intervals. Because of our approach to simulate dispersal, dispersal rates of particular species were a function of their relative abundances within local communities. This likely reflects natural dispersal mechanisms in metacommunities of passive dispersers.

The zooplankton community in each mesocosm was sampled at days 86 and 87 of the experiment using a Schindler Patalas (volume: 12 L; mesh size: 30 µm). Samples were preserved with acid lugol solution and individuals were counted and identified in the laboratory. Cladocerans were identified to species level, while copepods were only grouped into cyclopoids and calanoids. A minimum of 300 individuals from each sample was counted. For more details on the experimental design and protocol we refer to (Verreydt et al. 2012).

Statistical analyses

Effects of nutrient addition and dispersal on α-diversity

We used two-way analysis of variance to test for the effects of dispersal (3 levels) and nutrient addition (2 levels) on logarithmic transformed species richness and Shannon entropy [exponential of the Shannon index (Jost 2006)], two measures of α-diversity.

Calculation of total β-diversity and its components

Bray-Curtis dissimilarity coefficients based on species abundances were calculated for pairs of experimental communities and used as a measure of β-diversity. Next, we partitioned β-diversity into its two components, nestedness and replacement, using the *bray.part* function from the "betapart" statistical package (Baselga and Orme 2012) in R (R Core Team 2014). The output of this partitioning approach consists of three distance matrices representing: (1) β-diversity - i.e., Bray-Curtis; (2) the nestedness component of β-diversity; and (3) the replacement component of β-diversity. It is important to note that the nestedness component of β-diversity as calculated in this study is conceptually similar but yet different from the nestedness that is derived from multisite analyses (Leibold and Mikkelson 2002) [for a detailed explanation about the conceptual differences between "nestedness" and "the nestedness component of β-diversity", please see Baselga (2012)].

We decided to account for species abundances because quantitative data has been shown to provide more useful information on the mechanisms shaping diversity patterns within and among communities (Ulrich and Gotelli 2010, Baselga 2013). The "nestedness component of dissimilarity" in this study will therefore be > 0 in two situations: (1) when some species disappear from (or colonize) one site but not the other and/or (2) when some species become consistently less (or more) abundant in one site than in the other (Baselga 2013). The abovementioned situations can happen, for example, due to a change in the environment that directionally affects species abundances or extinctions. Likewise, the replacement component of dissimilarity will be > 0 when: (1) some species

completely replace each other and/or (2) when they change in relative abundances between sites (Baselga 2013). We removed one mesocosm (belonging to the HNUT – NODISP treatment) from the analysis because no species was present in this mesocosm at the end of the experiment.

Effects of nutrient addition and dispersal on β-diversity and its components

To test the independent and interacting effects of nutrient addition and dispersal on total *β*-diversity and its two components (i.e., nestedness and replacement) we performed multi-factorial PERMANOVA (Anderson and Walsh 2013) using the function "adonis" from the package "vegan" (Oksanen et al. 2016) in R. Multi-factorial PERMANOVA determines whether the level of dissimilarity among mesocosms from different treatments is higher than what would be expected by chance. Multi-factorial PERMANOVA generates tests of significance based on Monte Carlo randomizations, and also estimates R-square (R^2) values quantifying the explanatory power of the investigated factors (dispersal and nutrient levels in this study). We used these R^2 values to assess the relative magnitude of the dispersal and nutrient addition effects on total *β*-diversity and its two components. We ran PERMANOVA analysis using dispersal and nutrient levels as factors and as response variables the three distance matrices. Through this analysis, it was also possible to test for an interaction effect between dispersal and nutrients.

Effect of nutrient addition on β-diversity and its components within each dispersal level

In a second step, we carried out PERMANOVAs to evaluate the effect of the nutrient gradient (HNUT vs. LNUT mesocosms) on total *β*-diversity as well as on the nestedness and replacement components within each dispersal level separately. For this, we calculated total Bray-Curtis, the nestedness and replacement components within each dispersal treatment across the nutrient levels in the same way as described before. We then used those three distance matrices as response variables (one at a time) and nutrient level as a factor.

Effect of dispersal on β-diversity and its components within each nutrient level

In order to test whether the level of nestedness and replacement within each nutrient treatment (i.e., mimicking homogeneous landscapes) differ as a function of dispersal rates, we used PERMDISP. This analysis tests whether within group dissimilarity in species composition differs among groups (Anderson et al. 2006). To run PERMDISP we used the function "betadisper" in the "vegan" package using as response variables the nestedness and replacement distance matrices and dispersal as a factor. We then used the function "TukeyHSD" to test for significant pair-wise differences between dispersal levels.

RESULTS

Effects of nutrient addition and dispersal on α-diversity

Analyses of variance revealed that nutrient addition had a strong negative effect on species richness ($F = 100.783$, $p < 0.001$) and Shannon entropy ($F = 47.671$, $p < 0.001$), whereas dispersal had profound positive effects on species richness ($F = 30.013$, $p < 0.001$) and Shannon entropy ($F = 5.351$, $p = 0.006$) (see also Appendix S1). We found no indication for an interaction effect between dispersal and nutrient addition for species richness ($F = 2.662$, $p = 0.075$) or for Shannon entropy ($F = 0.397$, $p = 0.673$).

Effects of nutrient addition and dispersal on β-diversity and its components

Multi-factorial PERMANOVA revealed that the effect of nutrient addition ($_{adj}R^2 = 0.123$, $p = 0.001$) was twice as strong as the effect of dispersal ($_{adj}R^2 = 0.063$, $p = 0.001$) in affecting *β*-diversity (Fig. 2a). There was also a significant interaction between dispersal and nutrient addition on *β*-diversity ($_{adj}R^2 = 0.051$, $p = 0.001$) (Fig. 2a). When considering each component of *β*-diversity separately, we found a strong effect of dispersal ($_{adj}R^2 = 0.149$, $p = 0.001$) and a significant interaction between nutrient addition and dispersal rates on the nestedness component ($_{adj}R^2 = 0.061$, $p = 0.042$) (Fig. 2b). We found no effect of nutrient addition on the nestedness component ($_{adj}R^2 = -0.010$, $p = 0.907$). In contrast, nutrient addition had a strong effect on the replacement component ($_{adj}R^2 = 0.207$, $p = 0.001$) (Fig. 2c), and there was a significant interaction between nutrient addition and dispersal on the replacement component ($_{adj}R^2 = 0.056$, $p = 0.012$). We found no significant effect of dispersal on the replacement component ($_{adj}R^2 = 0.038$, $p = 0.055$).

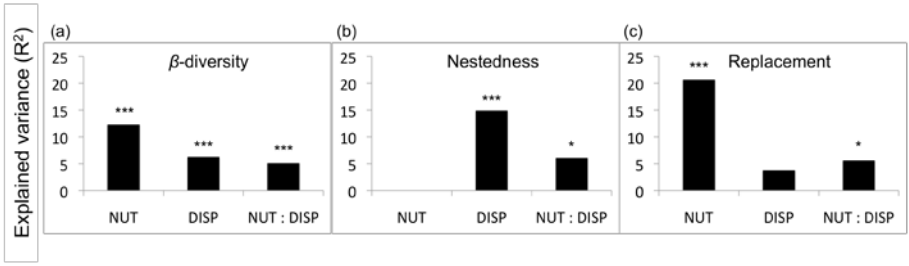

Figure 2. *Total amount of explanatory power (R^2; scaled to 100) provided by the nutrient treatment (NUT), the dispersal treatment (DISP) and their interaction (NUT : DISP) on β-diversity (a) and its components nestedness (b) and replacement (c). Effects of the treatments on β-diversity and its components were calculated based on PERMANOVA (see methods for details). ***: $p < 0.001$; *: $p < 0.05$.*

Effect of nutrient addition on β-diversity and its components within each dispersal level

One-way PERMANOVAs testing for the effect of nutrient concentration in each dispersal treatment separately revealed a significant effect of nutrient addition on the nestedness component but only in the unconnected mesocosms (NODISP) (Fig. 3a). In contrast, nutrient addition had no significant effect on the replacement component in the NODISP mesocosms, but was highly significant in the LODISP and, especially, in the HIDISP treatments (Fig. 3a). In the absence of dispersal, the nutrient addition treatment thus generated *β*-diversity solely via a nestedness pattern. In contrast, in mesocosms with low or high dispersal, *β*-diversity associated with the nutrient addition treatment was entirely structured according to a replacement

pattern. The effect of nutrient heterogeneity on *β*-diversity ranged from 9% in the NODISP treatment (R^2 = 0.091, $p < 0.005$) to 11% in the LODISP (R^2 = 0.108, $p < 0.001$) to 39% in the HIDISP treatment (R^2 = 0.393, $p < 0.001$).

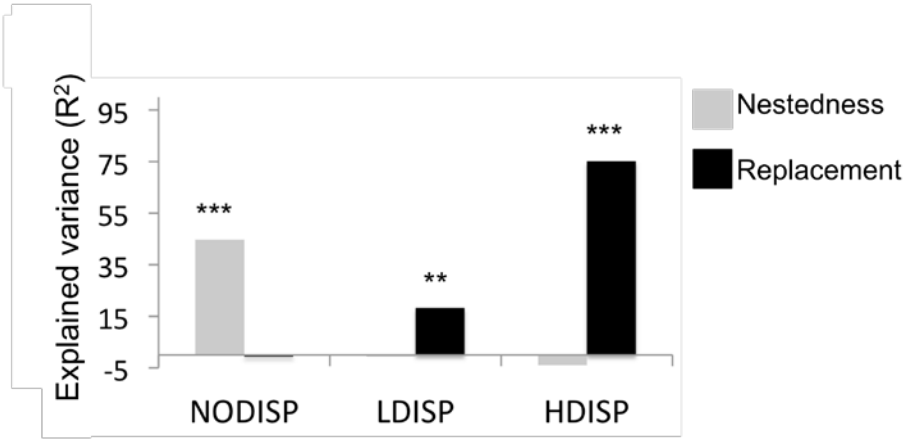

Figure 3. *Total amount of explained variance (R^2; scaled to 100) provided by the nutrient treatment on the nestedness component (grey bars) and on the replacement component (black bars) for each dispersal level separately. NODISP stands for the no dispersal treatment, LDISP refers to the low dispersal treatment, and HDISP to the high dispersal treatment. *** $p < 0.001$; ** $p < 0.005$.*

Effect of dispersal on β-diversity and its components within each nutrient level

PERMDISP revealed a highly significant difference in replacement within each nutrient treatment as a function of dispersal ($p < 0.001$ for both HNUT and LNUT treatments). Pair-wise comparisons revealed that the level of replacement within each nutrient treatment was highly significant between dispersal levels for both nutrient treatments, especially between the NODISP and HIDISP treatments (Table S3). In

contrast, we found no significant differences in the nestedness component within each nutrient treatment as a function of dispersal (Table S3). Ternary graphs revealed a sharp decrease in replacement and an increase in similarity with increasing dispersal rates within each nutrient treatment (see Fig. S2 in appendix S1). Specifically, average replacement decreased from 0.63 in NODISP mesocosms to 0.15 in HIDISP mesocosms exposed to high nutrient levels (HNUT). Similarly, average replacement decreased from 0.62 in NODISP mesocosms to 0.28 in HIDISP mesocosms with low nutrients (LNUT). There was a slight increase in the relative importance of the nestedness component with increasing dispersal rates, albeit this was not significant (see also Fig. S2 and Table S3 in supplementary information for all pair-wise comparisons between treatments).

DISCUSSION

Eutrophication due to nutrient loading and reduced dispersal owing to habitat fragmentation are global scale pressures that are increasingly affecting biodiversity patterns at multiple spatial scales (Kruk et al. 2009, Jeppesen et al. 2010, Hooftman et al. 2015). The results of our cross-factorial mesocosm experiment clearly demonstrate that nutrient enrichment strongly reduced local species richness and diversity, especially in isolated zooplankton communities. Consequently, the nestedness component of β-diversity was dominant across nutrient levels in unconnected mesocosms. In more connected communities, however, the loss of species due to nutrient additions was counteracted by dispersal, and this shifted the pattern of dissimilarity derived from nestedness to a pattern of dissimilarity derived from

species replacement. Thus, the interaction of nutrient addition and dispersal affected the components of β-diversity in divergent ways. This important interaction would never have been appreciated if the analyses had solely focused on total β-diversity patterns (Fig. 1 and Fig. 2a). In addition, we observed contrasting patterns for mesocosms belonging to the same nutrient levels (mimicking an environmentally homogeneous landscape) when exposed to different levels of dispersal. Here, we observed a sharp decrease in both β-diversity and species replacement with increasing dispersal levels, whereas the contribution of the nestedness component did not significantly change as a function of dispersal (Table S1). The contribution of the nestedness and replacement components to community dissimilarity across the dispersal levels for heterogeneous landscapes are fully in line with our predictions as outlined in Figure 1 if we assume that even the high dispersal treatment did not result in mass effects [which is in agreement with the pattern of strong species sorting reported for zooplankton in this dispersal treatment by Verreydt et al. (2012)]. Also the patterns of β-diversity as well as that of the nestedness and replacement components for homogeneous landscapes are largely in line with our predictions.

In the absence of dispersal, the negative effect of nutrient addition on local species richness along with a nested structure indicates that nutrient enrichment creates unsuitable conditions for a subset of zooplankton species. The observed pattern of directional species losses extends the conclusions of several field and experimental studies, which have demonstrated the negative effects of eutrophication on species diversity for many organism groups,

including zooplankton (Dodson et al. 2000, Declerck et al. 2007, Kruk et al. 2009, Declerck et al. 2011a). Nutrient addition impacted α-diversity negatively across all dispersal treatments (Fig. S1). However, this effect was entirely compensated by high dispersal rates (Fig. S1) resulting in a reduction of the nestedness component of β-diversity (Fig. 3a). This provides evidence for dispersal-mediated rescue effects in our mesocosm experiment (Hanski 1998) (see also Table S4 in Appendix S1). Increased dispersal also increased the importance of species replacement to β-diversity across the nutrient treatment. Specifically, dispersal fuelled species sorting by allowing species to spatially track suitable environmental conditions at the metacommunity scale, resulting in high complementarity in species composition (Cottenie and De Meester 2004). Previous studies have similarly demonstrated that immigration from the regional species pool has the power to counterbalance the detrimental effects of disturbance, such as high predation pressure, on zooplankton α-diversity and can enhance β-diversity (Shurin 2001, Howeth and Leibold 2010). We also found that β-diversity is enhanced in the presence of high dispersal rates across the nutrient levels. Through separating β-diversity into its nestedness and replacement components, however, we show that dispersal limitation and high dispersal both generate β-diversity in heterogeneous landscapes, but via completely different mechanisms. Specifically, high nutrient input results in species losses in unconnected mesocosms, thus generating β-diversity derived from nestedness. Conversely, high dispersal promotes species sorting across the nutrient levels and generates β-diversity via species replacement.

Contrary to our results, some observational studies found that increasing spatial connectivity diminished species replacement (i.e., spatial turnover) and increased nestedness in different organism groups, ranging from river fish to amphibians and mammals (McKnight et al. 2007, Melo et al. 2009, Leprieur et al. 2011). A potential explanation for these differences in outcome is spatial and temporal extent, since the studies above have focused at a biogeographic scale while ours focused at a metacommunity scale. At a biogeographic scale, reduced landscape connectivity likely promotes long-term evolutionary processes such as speciation and extinction, which might eventually result in large-scale turnover patterns due to differences in regional species pools (Leprieur et al. 2011). Despite previous theoretical studies on nestedness and turnover at the metacommunity scale, only recently have investigators started assessing the relative importance of dispersal and environmental processes on each component of β-diversity in empirical metacommunities, especially due to the development of new statistical tools (Baselga 2013, Legendre 2014, Baselga and Leprieur 2015). Using a different approach based on paired overlap and matrix filling to quantify nestedness *per se*, other studies reported patterns consistent with our results (Henriques-Silva et al. 2013, Bender et al. 2016). For instance, Henriques-Silva et al. (2013) found that harsher environmental conditions in combination with spatial isolation increased nestedness and reduced turnover in fish metacommunities. In another example, Bender et al. (2016) found that spatial isolation is an important driver of functional and taxonomic nestedness. Our findings similarly suggest that heterogeneous metacommunities are

shaped by ecological dynamics related to habitat filtering, competitive exclusion and dispersal (Shurin 2001, Bello et al. 2013, Spasojevic et al. 2014), which might create community dissimilarity via species replacement among more connected sites and via nestedness among isolated sites along disturbance gradients.

We found strikingly different patterns for β-diversity when we considered sets of mesocosms with the same rather than different nutrient levels, i.e. mimicking homogeneous landscapes. In homogeneous landscapes, dispersal limitation enhanced species replacement, whereas the nestedness component remained unchanged across the dispersal levels. The pattern of decreasing species replacement with increasing dispersal in homogeneous landscapes is in line with our predictions, and suggests that the same level of (high) dispersal promotes divergence in species composition in heterogeneous landscapes and community convergence in homogeneous landscapes (Leibold et al. 2004, Cottenie 2005, Logue et al. 2011). We observed that the contribution of the nestedness component was overall low and did not significantly change along the dispersal gradient, which is in agreement with our predictions (see Fig. 1 and Table S3 in Appendix S1). Changes in β-diversity as a function of dispersal were thus mainly derived from species replacements and not from nestedness (see also Fig. S2 in appendix 1). Whereas increasing dispersal enhances species complementarity via species sorting in heterogeneous landscapes, decreasing dispersal enhances species complementarity via stochastic drift in homogeneous landscapes. An open question is whether such high species replacement in unconnected and homogeneous landscapes can be

translated into functional complementarity. Although we have no data to further test this hypothesis, functional redundancy may be a dominant pattern in homogeneous and unconnected landscapes, meaning that different species with similar traits replace each other among local communities. The latter is likely to happen because species occurring in homogeneous landscapes have to be able to survive to similar environmental filters (Gianuca et al. 2014). Therefore, it is possible that landscape wide environmental homogenization resulting from a common environmental change, such as widespread eutrophication, selects for functionally redundant species, leading to rapid convergence of functional traits in metacommunities.

Increased nutrient loading and reduced dispersal rates owing to fragmentation are amongst the major drivers of species extinctions worldwide and can undermine ecosystem functioning and stability both in terrestrial and aquatic systems (Kruk et al. 2009, Symons and Arnott 2013, Hautier et al. 2015, Hooftman et al. 2015). Our study additionally suggests that nutrient loading and dispersal limitation synergistically enhance each other's effects on β-diversity. Our results confirm that conserving habitats based only on high levels of α-diversity is not sufficient, as it disregards the importance of variation in species composition among habitats (Angeler 2013, Tonkin et al. 2016). Because dispersal limitation interacts with eutrophication to create dissimilarity resulting mainly from nestedness in heterogeneous landscapes, we argue that sufficient habitat connectivity is crucial in allowing species to repopulate suitable sites and also to ensure high complementarity (replacement) among sites (Shurin 2001, Howeth

and Leibold 2010). However, the degree of connectivity in natural metacommunities depends not only on the spatial configuration of the habitat patches, but also on the dispersal mode and ability of the focal organism group (De Bie et al. 2012). As a result, different management strategies will often be needed to conserve α- and β-diversity of different organism groups. Based on our findings, it is conceivable that nestedness resulting from eutrophication will predominate among dispersal-limited groups and turnover will be more important for highly mobile groups within the same landscape (Tonkin et al. 2016). Future studies should, therefore, investigate how nestedness and turnover along disturbance gradients vary among organism groups that represent a range of dispersal abilities and strategies within the same landscape context.

The approach we applied here, experimentally manipulating dispersal and environmental conditions and teasing apart each component of β-diversity, was key to obtain new insights into how multi-scale assembly processes link local and regional diversity patterns at a metacommunity scale. It is important to note that in our setting dispersal rates always involved heterogeneous sources and were mainly a function of species relative abundances. The no dispersal treatment represents a situation in which a historically connected metacommunity becomes completely isolated, mimicking a fragmentation scenario. Also, the high dispersal treatment potentially started with higher species richness than the no dispersal treatment. While different dispersal mechanisms could have led to different conclusions (Grainger and Gilbert 2016), the type of dispersal we used in our experiment is likely a common situation in metacommunities

involving passively dispersed organisms, such as freshwater zooplankton (Lopes et al. 2016). Although experimental metacommunities represent only a simplified approximation of natural metacommunities, the results from our study are in clear concordance with those of some recent observational studies that used different methods to quantify nestedness and turnover [e.g., (Henriques-Silva et al. 2013, Bender et al. 2016)]. Additionally, our study extends the conclusions of previous observational studies by demonstrating divergent patterns between heterogeneous and homogeneous landscapes across dispersal scenarios, which was possible to accomplish only via experimentation. Therefore, our study provides a proof of principle of potential mechanisms structuring the nestedness and replacement components of dissimilarity in natural metacommunities, although some caution is recommended when extrapolating experimental findings to natural systems.

In summary, we demonstrated that nutrient enrichment negatively affects local zooplankton diversity and this creates community dissimilarity derived from nestedness in heterogeneous, unconnected metacommunities. However, we show that the balance between the nestedness and replacement components in our experimental metacommunity was completely altered by dispersal, as increasing dispersal reduced nestedness and resulted in high species replacement in heterogeneous landscapes. We also show that patterns of community dissimilarity derived from nestedness and replacement along dispersal gradients are completely altered in homogeneous environments. This also implies that environmental homogenization, which is likely to happen due to a strong common environmental

change such as global warming or widespread eutrophication (Moss et al. 2011), will strongly interact with landscape connectivity in its impact on the components of β-diversity. Taken together with previous analyses of this same dataset (Verreydt et al. 2012), our results indicate that sufficiently high dispersal in heterogeneous landscapes is fundamental for ecosystem functioning because it promotes biodiversity locally and optimizes the match between species and environment in metacommunities. Our study contributes novel insights on the mechanisms shaping β-diversity by demonstrating how environmental heterogeneity interplays with dispersal to create contrasting patterns of nestedness and replacement in metacommunities.

ACKNOWLEDGEMENTS

We thank Dino Verreydt for kindly sharing the dataset. Tad Dallas, Letícia Quesado, Caroline Souffreau and an annonymous reviewer provided valuable comments on an earlier version of this manuscript. ATG received a "Science Without Borders" PhD fellowship from CNPq-Brazil (grant number 245629/2012-2). This work was financially supported by KU Leuven Excellence Center funding PF/2010/07, Belspo IAP project SPEEDY and BiodivERsA project Tippingpond, nationally funded by Belspo.

SUPPLEMENTARY INFORMATION

Appendix S1. Additional figures and tables.

CHAPTER II

TAXONOMIC, FUNCTIONAL AND PHYLOGENETIC METACOMMUNITY ECOLOGY OF ZOOPLANKTON ALONG URBANIZATION GRADIENTS

(Manuscript conditionally accepted in Ecography)

Andros T. Gianuca, Jessie Engelen, Kristien I. Brans, Fabio Toshiro T. Hanashiro, Matthias Vanhamel, Edwin M. van den Berg, Caroline Souffreau and Luc De Meester

ABSTRACT

As human population size increases and cities become denser, several urbanization related selection pressures increasingly affect species assemblages in both terrestrial and aquatic habitats. Yet, it is not well known whether and how urbanization influences other facets of biodiversity, such as the functional and evolutionary composition of communities, and at what spatial scale urbanization acts. Here we used a hierarchical sampling design in which urbanization levels were quantified at seven spatial scales (ranging from 50 to 3200m radii). We show that urbanization gradients impose a strong selection pressure on zooplankton species traits, which in turn affects the phylogenetic composition of the entire metacommunity, but only when considering urbanization at the smallest spatial scale (50m radius). Specifically, small cladoceran species dominated in more urbanized ponds whereas large-bodied, strong competitors prevailed in less urbanized systems. We also show that trait and phylogenetic metrics strongly increase the amount of variation in β-diversity that can be explained by degree of urbanization, environmental and spatial factors. This suggests that the mechanisms shaping β-diversity in our study system are mediated by traits and phylogenetic relatedness rather than species identities. Our study indicates that accounting for traits and phylogeny in metacommunity analyses helps explaining seemingly idiosyncratic patterns of variation in zooplankton species composition along urbanization gradients. The fact that urbanization seems to act only at the smallest spatial scale suggests that correctly managing environmental conditions locally has the power to counteract the effects of urbanization on biodiversity patterns. The

multidimensional approach we explore here can be applied to other systems and organism groups and may help to understand how overall biodiversity changes in response to anthropogenic pressures and how this scales up to affect ecosystem functioning.

INTRODUCTION

Understanding how multi-scale anthropogenic pressures influence biodiversity patterns is a key goal in conservation biology and modern community ecology. As human population increases and cities become denser, several urban-related selection pressures have been shown to increasingly affect biodiversity patterns across spatial scales (Grimm et al. 2008). Increased pollution and high temperatures in urbanized areas, amongst others, can act as a filter on species composition and affect local community assembly (Arnfield 2003, Kaye et al. 2006). At the regional scale, urbanization can affect the spatial configuration of habitat patches and thereby influence dispersal rates among populations and communities (Urban et al. 2006). Integrating local and regional scale processes in analyses of metacommunity assembly along urbanization gradients can provide a useful framework to understand biodiversity patterns under realistic scenarios of global change.

Traditionally, metacommunity studies have quantified the relative importance of spatial and environmental gradients to explain variation in species composition among habitat patches (Cottenie 2005, Logue et al. 2011). An important source of criticism on this traditional approach is that it is blind to ecological similarities and differences among species (McGill et al. 2006). It has been increasingly recognized that differences in functional traits are key in determining diversity patterns within and among communities (Spasojevic et al. 2014, Liu et al. 2016). Therefore, accounting for species functional traits in metacommunity analyses may provide a more accurate understanding of biodiversity drivers (Spasojevic et al.

2014, Gianuca et al. 2016a). Yet, it is often unfeasible to *a priori* identify or quantify all relevant traits for multiple species at the metacommunity scale. An alternative approach to increase ecological realism is to account for species phylogenetic distances in metacommunity analyses, which may provide a more comprehensive representation of the multidimensional niches of species (Fig. 1) (Mouquet et al. 2012, Peres-Neto et al. 2012). This metacommunity phylogenetics approach assumes that functional differentiation among species is correlated with divergence time from a common ancestor, so that closely related species are expected to respond similarly to environmental and spatial gradients (Graham and Fine 2008, Wiens et al. 2010).

In freshwater systems, urbanization has been shown to affect taxonomic patterns of metacommunity structure (Urban et al. 2006, Johnson et al. 2013), but it remains an open question whether and how urbanization affects the distribution of traits and evolutionary history in freshwater metacommunities, and at what spatial scale urbanization acts. This is a pressing question because changes in the functional and phylogenetic composition of metacommunities in response to urbanization can influence fluxes of energy and organic matter through the food chain in aquatic systems (Thompson et al. 2015, Gianuca et al. 2016b) and thus affect the provisioning of ecosystem services to human populations. In freshwater zooplankton, body size is considered a strong response trait because it determines the position of species along gradients of productivity, fish predation pressure, pollutants, and temperature (Brooks and Dodson 1965, Moore and Folt 1993, Gianuca et al. 2016a), and some of these factors are

expected to change with urbanization. At the same time, zooplankton body size is also considered a key effect trait determining competitive strength and the capacity of top-down control of algae (i.e., larger species are superior grazers) (Brooks and Dodson 1965, Gianuca et al. 2016b). Therefore, size mediated species responses to urbanization will likely influence local species interactions and affect ecosystem processes, such as grazing pressure and herbivorous biomass production (Thompson et al. 2015, Gianuca et al. 2016b). Besides the important role of zooplankton body size in determining community assembly and ecosystem processes, other less explored functional traits such as filtration type and the degree in which species are associated with plants, can be key in shaping species interactions and species replacements along environmental gradients (Declerck et al. 2007, Vogt et al. 2013), for instance if urban ponds are characterized by reduced vegetation cover. Taking several traits into account could, therefore, provide better predictions of species responses to urbanization by better approximating species niches (Knapp et al. 2012), although in some instances confounding effects among contrasting traits can negatively influence the power of multi-trait indices (Butterfield et al. 2013, Gianuca et al. 2016a).

To the extent that measured and/or unmeasured functional traits are phylogenetically conserved, trait shifts along urbanization gradients will also affect the phylogenetic composition of entire metacommunities, with important implications for conservation of evolutionarily distinct taxa (Fig. 1a-b) (Helmus et al. 2010, Rolland et al. 2011, Faith 2015). However, when measured traits are labile, trait and phylogenetic patterns can be decoupled and provide

complementary insights on community assembly along urbanization gradients (Knapp et al. 2008). A stronger functional trait than phylogenetic response to urbanization would indicate that phylogeny does not capture information on traits that respond to urbanization. Conversely, a weaker functional trait than phylogenetic response to urbanization would indicate that measured traits are unresponsive whereas phylogenetic distances represent variation in unmeasured traits that strongly respond to urbanization (Fig. 1c-d). If species with similar functional traits replace each other from site to site along the urbanization gradient, then functional redundancy emerges at the metacommunity scale, resulting in higher taxonomic than functional (or phylogenetic) turnover.

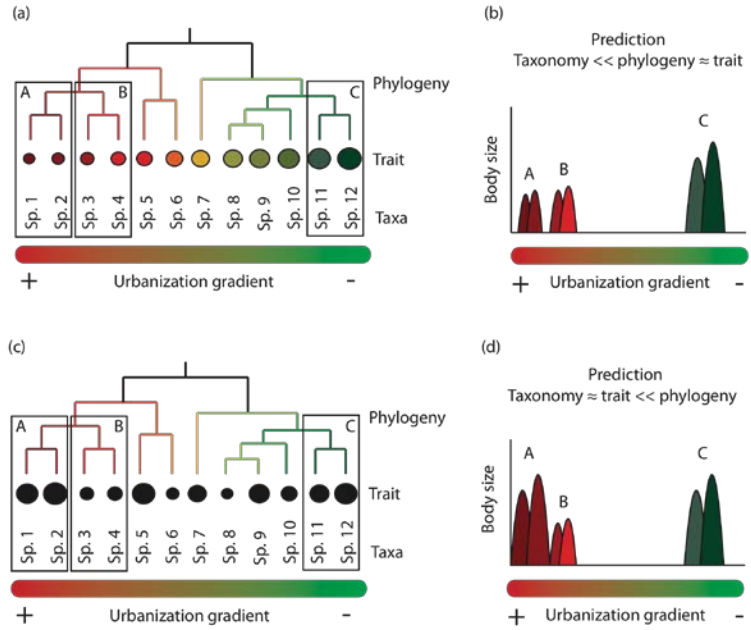

Figure 1. *Scheme depicting hypothetical scenarios of taxonomic, functional (trait), and phylogenetic beta-diversity patterns along an*

urbanization gradient. (a) Measured trait(s) are conserved along the phylogeny (e.g., body size, represented by the size of the circles) while phylogeny captures information in unmeasured trait(s) (represented by colored branches). Rectangles indicate three local communities (A; B; C) sampled along the urbanization gradient. There is a perfect match between trait, phylogeny and the urbanization gradient. At the taxonomic-level, all three local communities have maximum turnover (i.e., no species identities are shared among communities), so that the level of taxonomic turnover remains unchanged along the environmental gradient. (b) A strong shift in body size happens along the urbanization gradient (represented by communities A, B, and C), which is accompanied by phylogenetic turnover (colors) because body size and phylogenetic distances are correlated. (c) To the extent that measured traits are labile (e.g., size varies randomly along the phylogeny), phylogeny can be more informative than trait-based approaches if it better represents unmeasured traits that respond to the urbanization gradient (represented by matching environment-branch colors along the gradient). (d) Strong phylogenetic turnover, represented by a shift in colors of the occurrence curves of the different species along the urbanization gradient, is not accompanied by body size turnover if size varies randomly along the phylogeny. Note that another possible scenario is that in which phylogeny is uninformative whereas labile traits are (not represented here but see text for details).

To test whether and how urbanization can affect the taxonomic, functional and phylogenetic composition of zooplankton

metacommunities, we sampled 81 ponds and shallow lakes along an urbanization gradient and applied a multidimensional approach based on taxonomic, trait and phylogenetic data. For the trait-based approach we first considered a single key functional trait (i.e., body size) and then combined multiple traits in trait-based metrics. By using a hierarchical sampling design we assess at what spatial scale urbanization acts on different dimensions of biodiversity. We test three hypotheses. First, we hypothesize that environmental change in highly urbanized settings [e.g., due to increased pollution and higher temperatures (Arnfield 2003, Grimm et al. 2008, Kaye et al. 2006)], will lead to habitat filtering against specific traits, which may also lead to a phylogenetic signal in species turnover if the traits that are selected against are phylogenetically conserved. One potential such effect in cities, amongst others, is the urban heat island effects (Arnfield 2003, Oke 1973), which may select against large-bodied species (Brans et al. 2016). Second, we test the hypothesis that trait- and phylogeny-based approaches better explain metacommunity structure along urbanization gradients than the traditional taxonomic approach that treats all species as equally differentiated from each other. Third, we hypothesize that the three approaches (taxonomy, trait-based and phylogenetic) may capture different aspects of metacommunity structure, so that the relative importance of urbanization and specific environmental and spatial factors in explaining variation in community, trait and phylogenetic composition along the urbanisation gradient differs. The latter would imply that different conservation strategies are needed to preserve different dimensions of biodiversity.

METHODS

Study site selection

We used a stratified hierarchical design, which enabled us to differentiate between local and regional effects of urbanization. Based on an a priori GIS analysis (GIS software package ArcView GIS 3.2a, ESRI inc) of the percentage of built-up area, i.e. percentage of area covered by buildings (*Large-scale Reference Database*, Flanders Geographical Information Agency (Agiv 2013); scale: 1/250-1/5000), we sampled an urbanization gradient at both a regional (consisting of 3 by 3 km plots) and local scale (comprising of 200 by 200 m subplots). We defined three specific urbanization classes: high (>15% built-up area), medium (5-10% built-up area), and low (<3% built-up area) urbanization. Because built-up area only refers to buildings (not roads, parking lots, etc.), a 15% built-up area translates already into a very high level of urbanization (see Fig. S1: Appendix S1). For each class we then selected nine plots at the regional scale (i.e. 27 plots in total), and within each of the plots three ponds were chosen based on the three different urbanization classes at subplot level, using the same gradient in urbanization. So we sampled the same gradient in urbanization at the very local (200 x 200 m) as well as at a more regional (3 x 3 km) scale, and both measures of urbanization were shown to vary independently from one another (Engelen et al., under review). For the low urbanization level, an extra condition was added for the selection of plots: the area should contain at least 15% of biologically valuable land, to ensure that these low urbanized regions would be relatively high in natural land, and not only dominated by

intensive farmlands, which are typically also characterized by low urbanization but are heavily impacted by human activities [e.g. (Declerck et al. 2006)]. We selected ponds distributed over a polygon around the big cities of Ghent, Brussels, Antwerp, and Leuven (approx. 5000 km^2). Special care was taken that only land use was applied as selection criterion, and not the aspects of the ponds themselves (e.g., macrophyte cover, water transparency). We conducted a survey by means of electrofishing in most ponds in order to check whether we indeed selected only fishless ponds. Fish were observed in a minority of ponds, and only in very low numbers. Because the electrofishing technique is not entirely reliable to detect fish when they occur in very low abundances, we refer to the ponds as being fishless (most ponds are small and isolated and if not stocked are guaranteed fishless) or at most having very low densities of fish.

During May-July of 2013, a total of 81 small (<1ha) but permanent ponds were surveyed over a period of 42 days starting from end of May until beginning of July. To avoid interference from an effect of sampling time and any directional change in environmental condition associated with it, the order of sampling along the urbanization gradient was randomized over the different plot levels. Each day three ponds of an individual regional plot, and thus representing three different urbanization classes within a given plot, were sampled. Across days we randomized the plots to be sampled so that there was no bias with respect to region or urbanization level. We tested for an effect of sampling time on pond environmental conditions and species composition, and the effect proved to be non-

significant (see Appendix S1 for details and Fig. S1 for a map of the distribution of ponds).

Sample collection and analysis

Physical, chemical and morphometric pond variables were determined (see Appendix S1 for a detailed description of the sample collection protocol and the environmental variables that were assessed). Standard water characteristics were measured for each pond (pH, oxygen concentration, conductivity, water transparency). We analyzed water samples for the concentration of chlorophyll a, nutrients (total phosphorus and nitrogen), suspended matter, dissolved organic carbon, alkalinity, hardness and several major ions (calcium, chloride and sulphate ions). Water depth was measured with a graduated stick along an orthogonal transect of the pond. For each pond, a depth-integrated water sample was taken for zooplankton community in both the pelagic and littoral zones of the pond, by means of a tube sampler. A subsample of this volume of water was then filtered over a 64µm sieve; this subsample ranged from 20 up to 40L of water, depending on the zooplankton densities in the water. Samples were then fixated with formalin (7%) and stored in a 60 ml vial for posterior species identification.

For each sample a minimum of 300 individuals were counted and identified to species level. When no new species were found in the last 100 specimens, further identification of animals in the remaining sample was stopped. Densities were calculated as number of individuals per liter sampled. For the community analysis, we removed ponds (n = 9) in which less than two species were detected.

Land use data

The aim of the a priori pond selection, based on a hierarchical design, was to ensure that the ponds that were selected would cover the entire range of urbanization, and that urbanization level would not be confounded across spatial scales, but instead replicated independently for the regional and local scale. Our sampling design ensured that there were as many "rural" as "urban" subplots sampled within an urban or within a rural regional plot. This design was very important for us to be able to test for the independent effect of gradients of urbanization across spatial scales. However, rather than to just use these two spatial scales in our data analysis, we opted for a more detailed analysis across different spatial scales. To that end, we quantified percentage land use cover across seven different spatial scales (i.e. in radii of 50, 100, 200, 400, 800, 1600 and 3200 m around the pond). This approach is more refined and informative because it enables quantifying urbanization as a continuous variable across seven spatial scales instead of using a categorical classification of urbanization at two spatial scales (i.e., plot and subplot levels).

The percentage cover of land use types was estimated for circular areas with centre at the location of the ponds and a radius of 50m, 100m, 200m, 400m, 800m, 1600m, and 3200m from the pond. The land use types discerned were (1) built-up area; (2) arable land, (3) nature, (4) grassland, (5) cropland, and (6) forest. Coverage data were obtained through the application of the GIS software package ArcView GIS 3.2a (ESRI, Inc.). The topographical raster map of Flanders was used for built-up area (*Large-scale Reference Database*, Agiv 2013; scale: 1/250-1/5000), and for the remaining land use types

the land use coverage database of the Agency of Geographical Information Flanders [(Saeger et al. 2004) Biological valuation map, 2013, scale: 1/10000, 2013] was used.

Trait and phylogenetic distances among species

We extracted information from literature on three key zooplankton functional traits for all 23 species occurring in the metacommunity: body size, filtration type, and the degree in which species are associated with submerged plants; trait values and associated references can be found in Appendix S1. The first trait, body size, has been repeatedly shown to determine vulnerability to predators, competitive strength, and grazing performance of zooplankton, which makes this trait both a key response and effect trait (Brooks and Dodson 1965, Burns 1969, Gianuca et al. 2016b). Additionally, this trait is often positively correlated with life history traits (e.g. number of eggs produced throughout the lifespan) (Lynch 1980) and negatively related with physiological traits determining vulnerability to high temperatures and pollutants (Moore and Folt 1993). The other two traits, filtration type and plant association, determine resource partitioning and (micro)habitat segregation, respectively, and can be important for stabilizing coexistence among species that differ in competitive ability (e.g. small versus large species) (Barnett et al. 2007, Mayfield and Levine 2010). We first calculated body size distances among all species occurring in the metacommunity based on Euclidean distances using the "vegan" package (Oksanen et al. 2013) in the R version 3.2.4 (R Core Team 2016). Then, we calculated a trait distance matrix based on all three traits combined. Because body size is a continuous variable whereas the other traits are categorical, we

used Gower's distance to calculate this combined trait distance matrix (Borcard et al. 2011).

To assess phylogenetic distances among species, we calculated (cophenetic) phylogenetic distances among all species present in our metacommunity using the package "picante" (Kembel et al. 2010) in R. To do this, we used a recently published molecular phylogenetic tree for Cladocerans occurring in Belgium [for details see also Chapter III and supplementary information therein].

Statistical analysis

Phylogenetic signal in traits

The degree to which the measured traits show a phylogenetic signal was assessed by means of a two-step procedure consisting of a general Mantel test followed by a test based on the the Brownian motion evolutionary model (i.e., EM-Mantel; Debastiani and Duarte 2016). This adaptation of the Mantel test has appropriate type I error and strong power to detect phylogenetic signal for both continuous and categorical traits (Debastiani and Duarte 2016). The approach is based on two steps. First, a standard Mantel test is performed to assess the correlation between the phylogenetic distance and the functional trait distance matrices (here trait distances were calculated using Gower's distance based on all traits). If, and only if, this test is significant ($p < 0.05$), the second step is to test whether such correlation between phylogeny and traits is higher than what would be expected by chance given a specific evolutionary model (for more details, please see Debastiani and Duarte 2016). Here we used the Brownian motion evolutionary model, which assumes that differentiation in traits is

proportional to evolutionary time among species. Alpha values less than 0.05 would indicate that the measured traits are more conserved than what would be expected by such evolutionary model.

Phylogenetic and trait β-diversity

Phylogenetic *β*-diversity was calculated based on the phylogenetic distance matrix using mean pairwise phylogenetic dissimilarity among pairs of local communities (Swenson 2014), using the function COMDIST in R package "picante" (Kembel et al. 2010). This index calculates the mean phylogenetic distance among species in a pair of sites (Swenson 2014). We also used COMDIST to calculate functional *β*-diversity metrics based on both the body size distance matrix and the multi-trait distance matrix. The use of COMDIST for both trait and phylogenetic-based information allowed us to work with comparable metrics for trait and phylogenetic distances. Abundance values of species were considered when calculating both functional and phylogenetic *β*-diversity. Then, we applied Principal Coordinates Analysis (PCoA) over the COMDIST dissimilarity matrices individually (i.e., COMDIST based on phylogeny, COMDIST based on body size, and COMDIST based on the three traits combined) (Swenson 2014). The final product is a matrix of orthogonal PCoA eigenvectors, each of them describing phylogenetic and trait *β*-diversity patterns, which can be used as response variables in constrained ordinations (Anderson and Willis 2003, Duarte et al. 2012).

All eigenvectors generated by PCoA could in principle be used as descriptors of *β*-diversity patterns in successive analyses, but using all of them might introduce confounding effects in the analyses. As

each eigenvector represents an orthogonal synthetic variable of the gradients in β-diversity patterns, it is likely that some of these gradients are unexplained by the measured factors. We therefore make a selection of a subset of orthogonal eigenvectors that maximizes the fit between patterns of β-diversity (functional or phylogenetic) and the set of explanatory variables. The selection of the most appropriate number of PCoA eigenvectors to be used in subsequent analyses, was done by following the procedure proposed by Anderson and Willis (2003), which optimizes the fit between response and explanatory variables (for more details see Appendix S1).

Generating spatial descriptors

We used the geographical coordinates (UTM) of the sites to generate Principal Coordinates of Neighboring Matrices (PCNM) for Moran Eigenvector Maps (Griffith and Peres-Neto 2006). This technique allows assessing multiple spatial structures over the entire range of scales covered by the geographical sampling area. The first PCNMs generated in the analyses represent broader spatial structures, while the last ones cover finer spatial scales (Borcard and Legendre 2002). We retained for subsequent analyses only significant PCNMs associated with positive eigenvalues (n = 42).

Patterns along the urbanization gradient

To test how variation in species composition, functional traits and lineages were influenced by urbanization across spatial scales, we first selected the most parsimonious subset of urbanization variables to be used as explanatory variables. To do this, we used the method proposed by Blanchet et al. (2008), which is based on two criteria: (i)

the significance alpha level of 0.05 and; (ii) the adjusted-R^2 of the global model. Based on the resulting subset of explanatory variables describing the urbanization gradient, we performed standard regression analyses using as response matrices: (i) the PCoA eigenvectors describing patterns of functional *β*-diversity; (ii) the PCoA eigenvectors describing patterns of phylogenetic *β*-diversity; (iii) the Hellinger-transformed species abundance data; (iv) the PCoA eigenvectors describingsummarizing Bray-Curtis dissimilarities in species composition. The latter two response matrices are two alternative approaches for the taxonomic metacommunity analysis. The Hellinger-transformed species abundance data is the standard approach, whereas we also applied the approach based on PCoA eigenvectors describing dissimilarities in species composition to increase the similarity in procedures with the functional and phylogenetic metacommunity analysis. This was to avoid that differences in the amount of explained variation would be due to difference in the number of dependent variables in the analysis (please, see Apendix S1 for details on the taxonomic approach based on PCoA).

Quantifying the relative importance of urbanization, environment and space on β-diversity

In order to understand how the different dimensions of *β*-diversity (i.e., taxonomic, functional and phylogenetic) are influenced by more complex, multivariate gradients, we used variation partitioning. Variation partitioning allows disentangling the variation in community data (here also weighted by trait and phylogenetic distances) into a pure urbanization component, a pure environmental component, a

pure spatial component, the shared contributions between two or more factors, and an unexplained component of variation (Borcard et al. 1992). To run a traditional, taxonomic-based variation partitioning analysis we used as response matrix Hellinger-transformed species abundances per site (i.e., individuals/liter/pond). We additionally also used another taxonomic approach based on Bray-Curtis dissimilarity coefficients followed by PCoA (see Appendix S1 for details). This was done to check whether differences in the explanatory power between taxonomic and functional/phylogenetic approaches are not due to the number of response variables included in the model. Observed patterns of the two taxonomic approaches were very similar so that we report in the main text only the results obtained through a more traditional Hellinger-transformed approach (see Appendix S1 for the results of the Bray-Curtis analysis). To run variation partitioning on trait and phylogenetic data we used the selected eigenvectors describing phylogenetic and trait β-diversity patterns, respectively, as response variables. Before performing variation partitioning, explanatory variables were selected using forward selection (Blanchet et al. 2008). We selected a subset of urbanization, environmental and spatial variables for each biodiversity dimension separately (i.e., taxonomic, trait and phylogenetic). This allowed us to maximize the fit between explanatory and response data matrices while minimizing type I error. All analyses were run in R version 3.2.4 (R Core Team 2016).

Results

Phylogenetic signal in traits

Standard Mantel test revealed a strong correlation between trait and phylogenetic distances ($p < 0.001$). In addition, EM-Mantel indicated that the measured traits are more conserved along the phylogeny than what would be expected by a Brownian motion evolutionary model ($p < 0.001$).

Number of PCoA eigenvectors selected as response variables

Only the first PCoA eigenvector was selected as response variable for both functional (multi-trait and body size alone) and phylogenetic analysis. For the taxonomic approach, the first two PCoA eigenvectors were selected as response variables for the variation partitioning analysis (environment versus space), but no eigenvector was actually selected as response variable when analyzing taxonomic patterns exclusively along the urbanization gradient.

Patterns along the urbanization gradient

From the seven built-up measurements across spatial scales, only the percentage of built-up area at the smallest scale (50 meters) was selected through the forward selection approach as a significant predictor, and this for all biodiversity dimensions (i.e., taxonomic, trait and phylogenetic; $p < 0.05$; Table 1). We found that degree of urbanization was a weak albeit significant predictor of taxonomic turnover (i.e., variation in species composition; Hellinger-transformed) ($_{adj}R^2 = 0.04$, $p < 0.05$). There was, however, a strong turnover in body size along the gradient of built-up area ($_{adj}R^2 = 0.20$, $p < 0.001$). Specifically, larger species dominated in more rural ponds whereas smaller species prevailed in more urbanized systems (Fig. 2,

see also Fig. S2a in Appendix S1). Similarly, accounting for multiple traits in a trait-based approach revealed a significant functional turnover along the urbanization gradient ($_{adj}R^2$ = 0.13, p = 0.001). This shift in traits was accompanied by a strong phylogenetic turnover along the gradient of built-up area ($_{adj}R^2$ = 0.15, p < 0.001), with large Daphniidae species dominating in less urbanized ponds and small Chydoriidae species dominating in more urbanized systems (Fig. 2, see also Fig. S2b in Appendix S1).

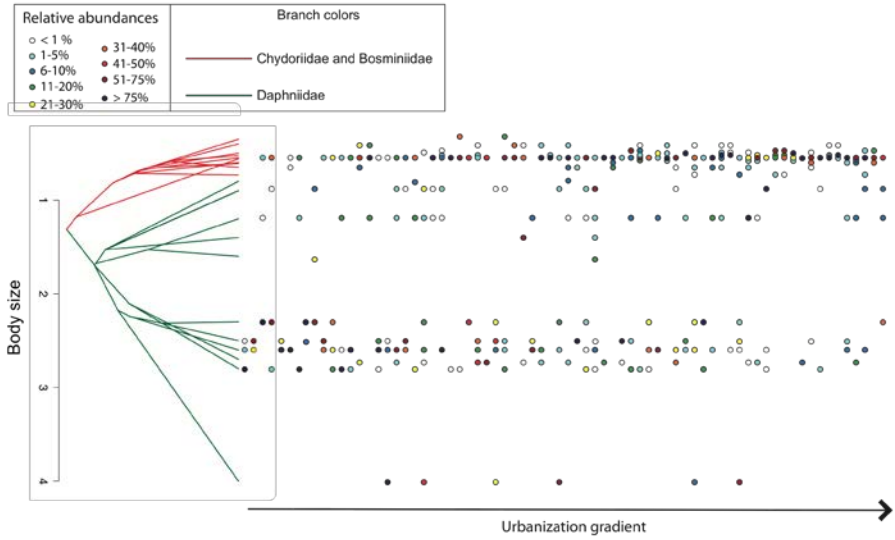

Figure 2. *Heat-map showing variation in species relative abundances along the urbanization gradient. Each of the communities of all the 72 sampled ponds is illustrated. Communities are ordered along a gradient of increasing degree of urbanization (built-up area) at 50 m radius. Species relative abundances are represented by colored circles and placed next to the evolutionary-traitgram. The evolutionary-traitgram posits the tips of the phylogeny according to a trait axis (here body size, ranging from 0.35 to 4.00 mm), while*

keeping the internal nodes proportional to evolutionary time (i.e., genetic distance in this example). For more details on the evolutionary-traitgram see Cadotte et al. (2013). Green branches are members of the Daphniidae family whereas red branches represent Chydoriidae and Bosminiidae (n=1) species.

Are trait and phylogenetic-based metacommunity approaches more informative than a taxonomic-based one?

Combining environmental, urban and spatial drivers of metacommunity assembly revealed that the informative power (i.e., total $_{adj}R^2$) obtained through variation partitioning was highest for the phylogenetic approach ($_{adj}R^2$=0.51), followed by the multi-trait (0.50), single-trait (0.43) and then taxonomic approach (0.12) (Fig. 3). Additionally, phylogeny, multi-trait and single-trait (i.e., body size) metrics significantly explained variation in the residuals of the best performing RDA model using environmental, spatial and urban-related variables on species composition (i.e., taxonomic approach; see Fig. S4 in Appendix S1). This finding indicates that accounting for traits and/or phylogeny improved predictions of environmental and spatial drivers of community assembly.

Are there differences in the relative influence of environmental, urban and spatial processes on each facet of biodiversity?

Variation partitioning revealed some differences in the relative importance of urbanization, environmental and spatial factors in explaining different β-diversity dimensions (Fig. 3). Pure environmental processes better explained multi-trait, phylogenetic, and taxonomic β-diversity patterns than the pure effects of

urbanization and spatial processes (Fig. 3). The pure effects of urbanization, environment and space similarly explained body size variation (Fig. 3). The pure effects of urbanization and spatial processes were still highly significant in explaining multi-trait, phylogenetic and taxonomic β-diversity (Fig. 3). Clearly, there was a large amount of shared effects between environmental and spatial processes in explaining all β-diversity dimensions. To a lesser extent, urbanization and spatial factors overlapped in their power to explain trait and phylogenetic β-diversity patterns. Finally, there was an overlap among all explanatory factors (i.e., urbanization, environmental and spatial factors) in explaining trait and phylogenetic, but not taxonomic, β-diversity patterns (Fig. 3).

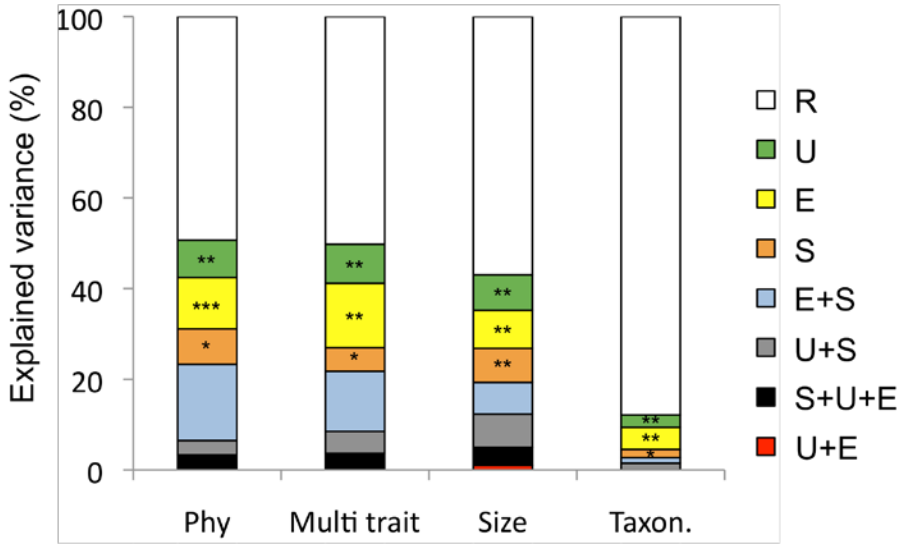

Figure 3. *Results of variation partitioning showing the contribution of urbanization [U], environmental factors [E], and spatial factors [S] to variation in different dimensions of biodiversity, as follows:*

phylogenetic composition (Phy), functional composition (Multi trait), body size composition (Size) and species composition (Taxon.). Components with a + sign indicate shared contributions of two or more factors. Variance explained refers to the $_{adj}R^2$ *(%). Three asterisks represent significant results p < 0.001; two asterisks p < 0.005; one asterisk p < 0.05.*

Despite of differences in explained variance, there was a large agreement among the environmental variables selected as important predictors of all β-diversity dimensions (i.e., taxonomic, single-trait, multi-trait and phylogenetic) (Table 1). The RDA analysis revealed that species composition varied mainly as a function of a gradient of total phosphorus, which was inversely related with the urbanization gradient and chlorophyll a on the first axis (Fig. 4). On the second axis, macrophyte infestation was negatively associated with pond area. Clearly, species of the Daphniidae family, which are relatively larger, pelagic and filter feeder species, were positively associated with total phosphorus and negatively associated with chlorophyll a and urbanization (Fig. 4). The opposite pattern was observed for the Chydoriidae species, which are relatively small, scrapers and plant associated. Percentage of built-up area for 50 m radius was the only urbanization variable explaining a significant variation in all biodiversity dimensions (Table 1).

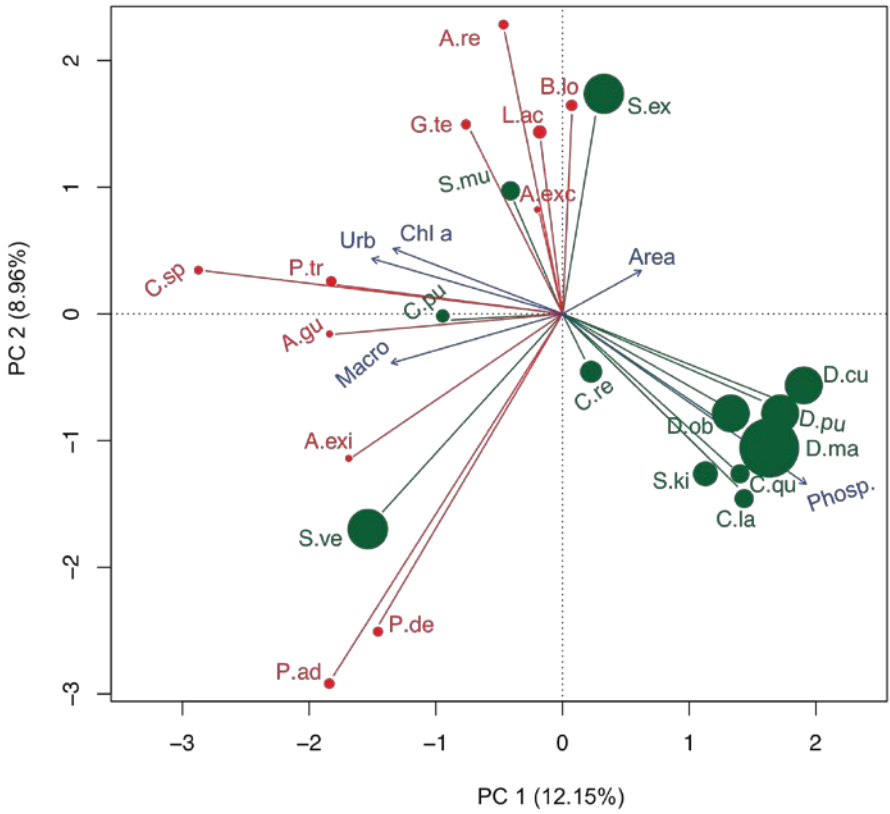

Figure 4. *PCA plot depicting the association between selected environmental and urbanization variables and species distributions. All Daphniidae species are shown in green, whereas all Chydoriidae and Bosminiidae (n = 1 species) are shown in red. The size of the circles is proportional to body size of the species. Explanatory variables are shown in blue. Urb = percentage of built-up area at the 50 m radius; Phosp. = total phosphorus; Macro = macrophytes; Chl a = chlorophyll a. Species acronyms are given in Appendix S1.*

Table 1. List of environmental, urbanization and spatial variables selected as significant predictors ($p < 0.05$) for each biodiversity dimension considered (i.e., taxonomic, single trait, multi-trait, and

phylogenetic dimension). Values refer to the adjusted-R^2; ns = not selected according to the two-step forward selection criteria as proposed by (Blanchet et al. 2008).

Selected explanatory variables	**Taxonomy**	**Body size**	**Multi-trait**	**Phylogeny**
Environmental model				
Total phosphorus	0.04	0.11	0.15	0.14
Macrophyte cover	0.02	ns	0.07	0.07
Chlorophyll a	0.02	0.07	0.08	0.08
Area (Log)	0.02	ns	ns	ns
Urbanization model				
Built-up (50 m)	0.04	0.20	0.13	0.15
Spatial model				
PCNM 24	0.04	0.12	0.13	0.14
PCNM 33	ns	0.07	0.07	0.07
PCNM 2	ns	ns	0.04	0.05
PCNM 5	ns	ns	0.05	ns
PCNM 28	ns	0.06	ns	ns

DISCUSSION

In this study we applied a multidimensional approach including taxonomic, trait, and phylogenetic data to study freshwater zooplankton metacommunity assembly along urbanization gradients. This integrated approach revealed a strong and highly significant body size turnover along the urbanization gradient. The pattern of body size turnover was accompanied by a significant phylogenetic and multi-trait turnover along the urbanization gradient, but only when urbanization was quantified at the smallest spatial scale considered (i.e., 50m radius). Specifically, small Chydoriidae species dominated in more urbanized systems whereas large Daphniidae species prevailed in less urbanized ponds. Taking together, the patterns of functional trait and phylogenetic turnover along the urbanization gradient support our first hypothesis that a shift in the dominant assembly processes from size-mediated competition in less urbanized systems to habitat filtering in more urbanized systems shapes trait and phylogenetic patterns along urbanization gradients (see also conceptual figure 1a-b). Noteworthy, urbanization effects on all biodiversity dimensions (i.e., taxonomic, functional and phylogenetic) were significant only at the smallest spatial scale considered (i.e., 50m radius). We also found that accounting for phylogenetic and trait distances in metacommunity analyses strongly increased the explanatory power provided by environmental, spatial and urban-related variables compared to a more traditional taxonomic approach. This supports the idea that trait and phylogenetic-based approaches better approximate ecological similarities and differences among

species and, therefore, have the power to provide more accurate predictions of the drivers of (meta)community assembly.

In the studied regional metacommunity of freshwater zooplankton, all three measured traits (body size, filtration type and the degree of macrophyte association) were highly conserved along the phylogeny. This resulted in highly concordant trait and phylogenetic patterns along the studied gradients. Different results were found for terrestrial plants along urbanization gradients in Germany (Knapp et al. 2008) and for ant metacommunities along gradients of forest habitat conversion (Liu et al. 2016), in which cases functional trait and phylogenetic patterns were completely uncoupled. The observed patterns of functional trait and phylogenetic turnover along urbanization gradients are in line with our predictions that harsh environmental conditions associated with urban areas filter out species from local communities [supporting hypothesis (i)]. Large zooplankton species are known to be more vulnerable to high temperatures and pollutants (Moore and Folt 1993, Symons and Shurin 2016). In our studied metacommunity of ponds and shallow lakes, there is evidence for increased water temperatures in ponds located in more urbanized areas (Brans et al. 2016). The higher temperatures in more urbanized ponds and perhaps also other unmeasured anthropogenic pressures, such as pollution, could have acted as a filter and selected against larger species in our study system, thus resulting in significant pure effects of urbanization on body size and phylogenetic turnover (Figs. 3 and 4). Conversely, a number of studies have reported that competition tends to be stronger in more benign environments (Mayfield and Levine 2010,

HilleRisLambers et al. 2012). Competition among zooplankton species is often size mediated, as numerous studies have demonstrated that large zooplankton species are stronger competitors and tend to competitively exclude smaller species from local communities (Brooks and Dodson 1965, Dodson 1974, Shurin 2001, Symons and Shurin 2016). Hence, it is likely that increased competition in less urbanized ponds mediated the dominance of large species in those systems, whereas small species that are weaker competitors increased in abundance in more urbanized systems, owing to competition release mediated by habitat filtering involving the elimination of larger species in more urbanized systems.

Previous studies have suggested that accounting for traits and/or phylogenetic distances among species could provide more accurate predictions of environmental and spatial drivers of community assembly by approximating species niches (McGill et al. 2006, Peres-Neto et al. 2012, Spasojevic et al. 2014). We tested this idea along urbanization gradients and found that accounting for trait and phylogenetic distances among species increased the explanatory power of the analyses substantially [supporting hypothesis (ii)]. First, when we analyzed β-diversity patterns exclusively along the urbanization gradient, we found a stronger body size turnover than a phylogenetic, multi-trait, and taxonomic turnover (Table 1). This indicates that species responses along the urbanization gradient were mainly size mediated and that including other traits or phylogeny in the analysis dilutes the explanatory power of the analysis. Other studies have similarly reported that accounting only for the best single trait associated with a given environmental gradient or spatial scale

can maximize the power of community analysis compared to multi-trait analysis (Butterfield et al. 2013, Gianuca et al. 2016a). This likely reflects confounding effects among traits that are associated with different structuring mechanisms.

Interestingly, when we analyzed β-diversity patterns along more complex, multivariate gradients, we observed that the explanatory power of the analyses increased with metric complexity from single-trait to phylogenetic and multi-trait indices. The fact that in our study the power of the analyses increases with metric complexity suggests that the measured traits and phylogenetically conserved traits actually reinforce each other's signal along the sampled environmental and spatial gradients. This suggests that different species with similar trait combinations respond in similar ways along the complex environmental and spatial gradients, so that directly accounting for several traits instead of only for species identities maximizes the correlation between measured environmental factors and β-diversity patterns. Therefore, our results indicate that apparently idiosyncratic species responses to the sampled gradients are in fact largely trait- and phylogenetically mediated. This was also supported by our residual analysis (see Fig. S3 in Appendix S1). This indicates that urbanization mainly affects species composition through body size constraints, while phylogeny and several other traits better represent species responses along more complex environmental gradients in our dataset. This was not the pattern observed by Gianuca et al. (2016a; Chapter III) along gradients associated with agricultural land use intensity, in which phylogenetic approaches were always outperformed by approaches that account only for the best single

zooplankton trait. Yet, Gianuca et al. (2016a; Chapter III) similarly found that taxonomic based approaches are less effective in revealing environmental and spatial drivers of metacommunity organization than approaches informed by traits and phylogeny. The reduced effectiveness of taxonomic-based analysis was not dependent on the approach used (i.e., Hellinger-transformed or Bray-Curtis followed by PCoA), suggesting that trait and phylogenetic data indeed better represent species responses along urbanization, environmental and spatial gradients [see also Appendix S1 and Gianuca et al. (2016a)].

Despite differences in overall explanatory power provided by variation partitioning analysis on taxonomic and trait or phylogenetic approaches, the same subset of environmental and urban-related variables was repeatedly selected as the most important drivers of all β-diversity dimensions [at odds with hypothesis (iii) for this data-set; Table 1]. This subset of variables included total phosphorus, pH, macrophytes, and percentage of built-up area for the 50 m radius. Particularly, we found that total phosphorus was consistently the best environmental predictor of all β-diversity dimensions and that Daphniidae species were positively associated with phosphorus (Fig. 4). In general, Daphniidae are larger than Chydoriidae and previous studies have already reported a positive relationship between phosphorus and zooplankton body size (Tillmann and Lampert 1984, Dodson et al. 2000). However, it is not entirely clear if the negative association between small Chydoriidae species and phosphorus in our dataset is entirely size-mediated, for instance reflecting competitive exclusion by large Daphniidae species (Dodson et al. 2000), or rather dependent on other traits that are conserved along the phylogeny. For

instance, all Chydoriidae species are scrapers and macrophyte associated (Barnett et al. 2007) and we found a negative relationship between macrophytes and phosphorus. Consequently, the negative association between Chydoriidae and phosphorus could also be driven by the absence of macrophytes in ponds with very high nutrient concentrations. The genus *Simocephalus* is the only taxon of the Daphniidae that evolved to be plant associated. The two *Simocephalus* species in our dataset were not positively associated with available phosphorus, whereas other similar-sized *Daphniidae* species do show a strong positive response to the phosphorus gradient. Therefore, our analysis reveals that the association between phosphorus and body size might be, at least in part, driven by additional correlated traits that are conserved along the phylogeny, such as the degree to which species are plant associated and their filtration type (e.g, scrapers or filter feeders). The latter helps to explain why the power of the analysis increases from pure size information towards more complex multi-trait and phylogenetic information along multiple environmental gradients in our dataset.

The pure effect of spatial processes on trait and phylogenetic β-diversity suggests that dispersal limitation or unmeasured but spatially structured environmental variables were affecting species distributions. The lack of spatial signal on taxonomic patterns suggests that dispersal limitation or the association with unmeasured environmental variables is probably mediated by the measured traits (Saito et al. 2015), of which body size may be a strong candidate. The strong pure effect of urbanization on functional traits and phylogenetic patterns indicates that some unmeasured variables potentially change

along the urbanization gradient and affect β-diversity. Good candidate variables may be temperature regimes (which was not measured in this study through time, but as a single measure during the sampling day) and different types of pollution, such as heavy metals and pesticides.

Local environmental processes were overall the most important drivers of β-diversity patterns either as pure effects or shared with other factors (Fig. 3). This indicates that correctly managing environmental conditions within ponds has the power to counteract selection pressures imposed by regional-scale urbanization on biodiversity. Moreover, the only spatial scale at which urbanization was actually relevant in determining all biodiversity dimensions was 50 m radius around the ponds (i.e., the smallest spatial scale considered). This is in agreement with earlier studies (Declerck et al. 2006) and implies that buildings in the immediate vicinity of the ponds affect zooplankton species distributions (Brans et al. 2016). Other studies reported evidence for heat accumulation due to the low reflectance of urban structures and impervious surfaces, thereby inducing runoff of hot water into streams and ponds (Somers et al. 2013, Taleghani et al. 2014). Urban green space such as parks are known to alleviate the urban heat island effect, as they provide a microclimate thanks to the evaporation and shading effect of trees (Hamada and Ohta 2010). In this way they create a so-called Park cool-island (Kleerekoper et al. 2012), with the size of the green areas determining the magnitude of the cooling effect (Chang et al. 2007, Li et al. 2011). Given that body size is a very responsive trait along urbanization gradients and that large-bodied zooplankton are superior

grazers that may be more efficient in reducing algal biomass, a useful conservation strategy is to create a belt of green area around each urban pond, which can potentially alleviate the heat-island effect and mitigate the physiological stress imposed by high temperatures on large zooplankton species (Brans et al. 2016, Moore and Folt 1993). Although we used a correlative approach, our study actually provides a glimpse on the potential functional consequences of body size shifts along urbanization gradients. For instance, we observed a negative correlation between phosphorus concentration and chlorophyll a along the urbanization gradient, which may seem counterintuitive at first sight because phosphorus is a key nutrient inducing phytoplankton production. However, such a negative correlation potentially reflects the predominance of larger, superior grazer species in ponds with high phosphorus concentration, which results in a reduction of chlorophyll a in those systems via top-down control of algae (Fig. 4) (Gianuca et al. 2016b).

Our analyses are based on a single sampling campaign, and seasonal variation was not covered. Our analyses may therefore underestimate the influence of urbanization on cladoceran communities earlier or later in the season. We did not, however, observe any influence of sampling date on community composition in our data set (May-July; see Appendix S1), and others have reported that cladoceran communities tend to be relatively stable during summer months [e.g. (Boven and Brendonck 2009)]. Therefore, we are confident that our analysis captures relevant patterns of strong habitat filtering on cladoceran zooplankton trait composition along urbanization gradients that at least hold for the summer season. Our

results are also in line with earlier work on zooplankton community structure in urban ponds that were carried out in different regions and seasons or involved monitoring through time (Walseng et al. 2006, Pinel-Alloul et al. 2013).

In summary, we found that increasing urbanization is not only affecting species composition in ponds but it is mainly and largely altering the functional composition and the evolutionary history of the entire metacommunity. Largely in line with our predictions, more natural systems were dominated by large, superior competitor species, whereas increased urbanization resulted in a dominance of small cladocerans. This suggests that urbanization affects community assembly via strong habitat filtering against large zooplankton species, which are in general of the Daphniidae family in our study. These findings have important implications for conservation and the maintenance of ecosystem functions under a scenario of increasing urbanization, especially because large-bodied species, which are stronger competitors and grazers on algae, are the first to be eliminated from highly urbanized ponds. Therefore, we suggest that in order to preserve high levels of ecosystem functioning in urbanized ponds, conservation strategies should target environmental conditions that guarantee the persistence of larger zooplankton species in urban ponds. Noteworthy, the vast majority of the sampled ponds in this study were fishless, so our results suggest that removing fish from city ponds will not be enough in order to preserve larger zooplankton species in urban ponds. Thanks to our hierarchical sampling design, we were also able to demonstrate that the negative effect of urbanization on larger zooplankton species only occurs at the smallest

spatial scale considered (i.e., 50 m radius) and rapidly disappears when urbanization is measured at larger spatial scales. Therefore, maintaining green areas around city ponds can potentially guarantee the persistence of larger zooplankton species in those systems and thus the maintenance of high levels of ecosystem processes such as top-down control of algae and herbivorous biomass production. The multidimensional approach we applied here is especially useful to understand how different facets of biodiversity will respond to increasing anthropogenic pressures, such as those associated with urbanization.

ACKNOWLEDGEMENTS

We thank all partners of the SPEEDY project for their collaboration. We are also grateful to Carla Denis, Melissa Schepens, Pieter Lemmens and Io Verdonk for their contribution with fieldwork and sample analysis. We thank Vanderlei Debastiani for his support concerning the statistical analysis. Steven Declerck, Pieter Lemmens and two anonymous reviewers provided valuable comments on an earlier version of this manuscript. ATG and FTTH received full PhD scholarships from Brazil under the program “Science Without Borders - CNPq” (grant number 245629/2012-2 and 245968/2012-1, respectively). JE and MV enjoyed a PhD fellowship of the IWT Flanders (IWT - project number 121625 and 131569, respectively). KIB is supported by Fund for Scientific Research - Flanders (FWO Vlaanderen, application number 11N5116N). This work was financially supported by Belspo IAP P7/04 project SPEEDY and by KU Leuven Research Fund excellence financing P/2010/07.

SUPPLEMENTARY INFORMATION

Appendix S1. Detailed description on methods and supporting statistics.

CHAPTER III

INTEGRATING TRAIT AND PHYLOGENETIC DISTANCES TO ASSESS SCALE-DEPENDENT COMMUNITY ASSEMBLY PROCESSES

[Modified from Ecography (2016), doi: 10.1111/ecog.02263]

Andros T. Gianuca, Steven A. J. Declerck, Marc W. Cadotte, Tom De Bie, Caroline Souffreau and Luc De Meester

ABSTRACT

Biodiversity is structured by multiple mechanisms that are dependent, at least in part, on ecological similarities and differences among species. Integrating traits and phylogenies in diversity metrics may provide deeper insight into community assembly processes across spatial scales. However, different traits are influenced by processes at different spatial scales, and it is not clear how trait-spatial scale mismatches skew our ability to detect assembly patterns. An additional complexity is how phylogenetic distances, which might capture unmeasured traits, reflect spatially dependent processes. Here we analyze a freshwater zooplankton dataset from 91 ponds and show that different traits are associated with processes at different spatial scales. We first assessed the response of individual traits to processes at both α- and β-scales, and then quantified the power of different combinations of traits and phylogenetic distances to reveal environmental and spatial drivers of α- and β-diversity. We found that explained variance was maximised when we accounted for environmental and spatial drivers with single, but different traits for α- and β-diversity. Using the most appropriate trait for each spatial scale outperformed phylogenetic information, but phylogenetic information outperformed the same traits when these were used at the wrong spatial scale, and all outperformed taxonomic analyses that ignore trait and phylogenetic information. We demonstrate that accounting for species' similarities and differences provides important information about dominant assembly mechanisms at different spatial scales, and that phylogeny is especially useful when measured traits are uninformative at a given spatial scale or when there is lack of trait data. Our study also indicates, however, that trait-scale mismatches

among phylogenetically conserved traits may affect the performance of phylogenetic indices compared to indices that account only for the best single trait at each spatial scale.

INTRODUCTION

Traditionally community ecologists have quantified biodiversity using metrics based on species identities alone, which are blind to ecological similarities and differences among species. Increasingly, trait and phylogenetic-based metrics are being used to link diversity patterns to potential mechanisms operating within (α-diversity) and among (β-diversity) communities (Cavender-Bares et al. 2009, Leibold et al. 2010, Peres-Neto et al. 2012). The use of phylogenies in ecology assumes that closely related species resemble each other more in their traits than do distantly related species, and that phylogenetic indices would synthetically represent species multidimensional niches (Wiens et al. 2010). Phylogenetic measures are therefore expected to provide a richer understanding of both α- and β-diversity drivers than analyses based on a few traits or species identities alone (Graham and Fine 2008, Mouquet et al. 2012, Weinstein et al. 2014).

There are, however, two potential limitations associated with approaches that rely exclusively on phylogeny and disregard trait information. The first and more obvious one is that traits can be labile, so that phylogeny may not really capture species ecological similarities and differences (Gerhold et al. 2015). The second limitation, which is often neglected in community ecology studies, is that confounding or idiosyncratic effects among phylogenetically conserved traits can actually affect the performance of phylogenetic indices (Trisos et al. 2014). This can happen when traits have contrasting responses along environmental gradients, thus canceling each other's signal (Spasojevic and Suding 2012, Butterfield et al. 2013). With this type of complexity, it seems logical that accounting separately for traits associated with different assembly processes can

provide superior explanatory power in analyses of community assembly than phylogeny [i.e., because phylogenies integrate variation in different traits simultaneously (Trisos et al. 2014)].

Paralleling diversity patterns along multiple environmental gradients, biodiversity patterns at different spatial scales (e.g. within and among communities) can also be controlled by different axes of niche space (Silvertown et al. 2006, Ackerly and Cornwell 2007, Messier et al. 2010). Species coexistence within communities is mainly determined by traits involved in resource exploitation, microhabitat use, or diet (Ackerly et al. 2006). Following Pickett and Bazzaz (1978), Silvertown et al. (2006) used the term α-niche to refer to this local scale dimension of niche differentiation. Conversely, at larger spatial scales species are sorted based on their habitat preferences or environmental tolerances (Cavender-Bares et al. 2006, Cavender-Bares et al. 2009). The term β-niche (Silvertown et al. 2006) has been used to refer to this larger-scale component of niche differentiation as it defines the positioning of species along environmental gradients. Accounting separately for α- and β-traits in analyses of community assembly may therefore enrich our understanding of biodiversity drivers across spatial scales and it is a fundamental problem of approaches that account solely for phylogeny that one cannot separate traits associated with different environmental axes or spatial scales.

Previous trait and phylogeny-based community ecology studies have either been limited to a single spatial scale or have not distinguished α- and β-niche traits in multi-scale analyses [e.g., (Cavender-Bares et al. 2004, Vogt et al. 2013, Weinstein et al. 2014)],

thus ignoring potential confounding effects among traits. Because α- and β-niche traits present different responses to processes at different spatial scales (Silvertown et al. 2006), combining those traits may reduce explanatory power due to a trait-spatial scale mismatch. The latter highlights an important challenge in trait and phylogenetic-based community approaches. Using too few traits can result in a lack of power because the selected traits do not represent the multitude of processes affecting community assembly across spatial scales (Lefcheck et al. 2014, Kraft et al. 2015). Conversely, using several traits or phylogenetic information can introduce confounding effects in the analysis if these traits are associated with different structuring mechanisms (Spasojevic and Suding 2012, Trisos et al. 2014).

As a step forward, we here propose a conceptual framework for integrating trait and phylogenetic data into community analyses aiming at maximizing analytical performance and enhancing the interpretation of environmental and spatial drivers of community assembly and turnover. We propose that measured traits should be evaluated separately and then classified *a posteriori* based on their responses to α- and β-scale assembly processes. This avoids *a priori*, artificial trait categorization and can be easily done because α-niche trait(s) are by definition more informative locally whereas β-niche trait(s) are more informative at a regional scale (Fig. 1) (Silvertown et al. 2006). We then propose to integrate α- and β-traits separately with phylogenetic distances through the functional-phylogenetic approach developed by Cadotte et al. (2013). One important aspect of the functional-phylogenetic approach is that it allows differential weighting of traits and phylogeny so that investigators can assess the

relative contributions of the two measures to explaining community patterns (Cadotte et al. 2013). While originally used to study α-diversity, we here extend its application to study β-diversity as well, thus achieving straightforward comparisons across spatial scales. This extension allows us to better understand if trait and phylogenetic approaches are equally informative across spatial scales or rather complement each other. The power of our approach is that one can account for α- and β-niche traits separately and use phylogenetic information as a benchmark to assess the performance gain obtained by this more encompassing representation of species multidimensional niches.

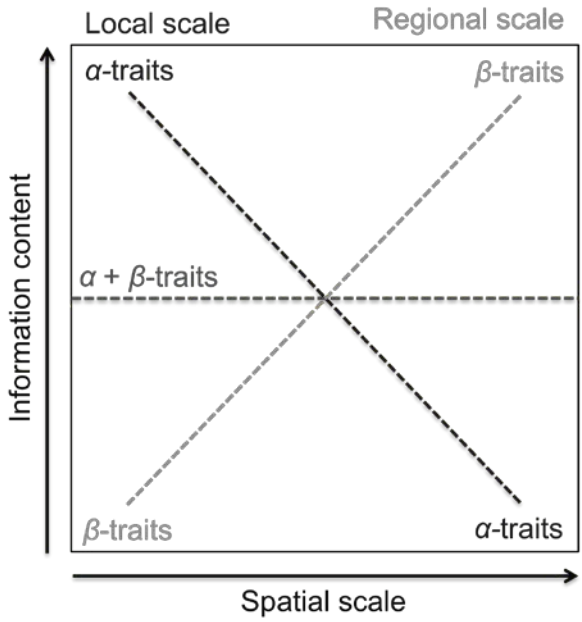

Figure 1. *Information on α-niche traits provides increased explanatory power on drivers of α-diversity but will be of no or low information content for patterns of β-diversity. Conversely,*

information on β-niche traits provides increased explanatory power on drivers of β-diversity but will be of no or low information content for patterns of α-diversity. Combining both types of traits results in reduced information content compared to only including the trait matching the spatial scale of interest, but provides increased information compared to only including traits that are associated with processes at the other spatial scale. The added value of including phylogenetic information over purely taxonomic metrics or trait-based approaches will depend on whether α- or β-niche traits are phylogenetically conserved. If phylogeny captures information on both α- and β-niches then phylogenetic-based metrics will behave similarly to trait-based metrics that combine α- and β-niche traits.

To illustrate our conceptual framework we integrated traits and phylogenetic information to assess scale dependent assembly patterns in a regional zooplankton metacommunity involving 91 ponds and shallow lakes, using phylogenetic information as a benchmark to assess the informative power of different traits at different spatial scales. Specifically, we applied our framework to test three key ideas: (i) depending on the spatial scale considered (α-scale, β-scale), functional diversity in metacommunities is structured by different environmental or spatial processes that relate to different traits; (ii) different trait responses to α- and β-scale assembly processes affect the performance of combined trait indices in community analyses; and (iii) phylogenetic distances are informative at both spatial scales and can be used as a substitute for uninformative or missing traits, but might be outperformed by trait-based analyses that maximize the

match between trait and spatial scale. We also compared trait and phylogenetic patterns with more traditional taxonomic-based approaches to assess the performance gain obtained by integrating traits and phylogeny into community analyses. We note that there are likely to be a multitude of traits involved in α- and β-niche processes. However, as a means of testing our proposed conceptual framework, we focus here on two key zooplankton traits that are expected to behave differently across spatial scales.

METHODS

Functional-phylogenetic framework for α- and *β-diversity analyses*

Our framework is based on the idea that different traits can be integrated with phylogeny in α- and β-diversity analyses to maximize the match between relevant niche dimensions and spatial scales. Functional trait (FDist) and phylogenetic distance (PDist) matrices can be combined into functional-phylogenetic distance matrices (FPDist) following Cadotte et al. (2013). This can be done by weighting the independent contributions of PDist (a) and FDist (1-a) to FPDist as follows: $FPDist = [aPDist^p + (1 - a)\ FDist^p]^{1/p}$, where p represents a p-norm distance that is analogous to Euclidean distance when p = 2 (Cadotte et al. 2013). Functional-phylogenetic distance can thus be viewed as a compromise between functional and phylogenetic information, which can be tuned with the parameter, a. When a = 1, FPDist only represents the pure phylogenetic distances (i.e. is equal to PDist) and when a = 0, FPDist represents the pure functional trait distances (i.e. is equal to FDist). Intermediate values of parameter a

imply that both sets of information are used and their relative contribution is weighted by *a*.

Integrating α- and β-traits with phylogeny in α- and β-diversity analyses provides a strong diagnostic tool to scale the explanatory power of specific traits to processes at different spatial scales as well as to account for unmeasured, phylogenetically conserved traits associated with each spatial scale (Fig. 2). Theoretically, there are several possible outcomes in trait and phylogeny-based community analyses across spatial scales, which ultimately depend on which traits are measured and which traits are labile or conserved (Fig. 2) [see also (Cadotte et al. 2013)]. First, confounding effects among α- and β-traits may affect the performance of phylogenetic-based approaches if both α- and β-niches are conserved along the phylogeny. Therefore, it may be more powerful to account directly for α- and β-traits separately in analyses of α- and β-diversity, respectively, than accounting solely for phylogeny (Fig. 2a-b). This pattern can be detected by an increase in explanatory power of the analysis towards pure trait information (i.e., low values of the parameter *a*), with different traits responding to processes at different spatial scales. However, α- and β-niche traits may evolve under different rates (Ackerly et al. 2006, Silvertown et al. 2006), so that phylogeny can be uninformative at a given spatial scale (i.e., due to labile traits) and, at the same time, highly informative at another spatial scale (i.e., due to phylogenetic signal in unmeasured traits) (Fig. 2c-d). Additionally, phylogeny may be more informative than the measured traits at a given spatial scale if it better represents variation in unmeasured traits associated with that spatial scale (Fig. 2 e). Such pattern will lead to an increase in informative power towards

pure phylogenetic information (i.e., high values of the parameter *a*). Finally, traits and phylogeny may complement each other when measured traits are highly informative but labile and phylogeny represents variation in additional, unmeasured traits that are highly informative at the given spatial scale [see also (Cadotte et al. 2013)]. This will lead to an increase in informative content for intermediate *a*-values.

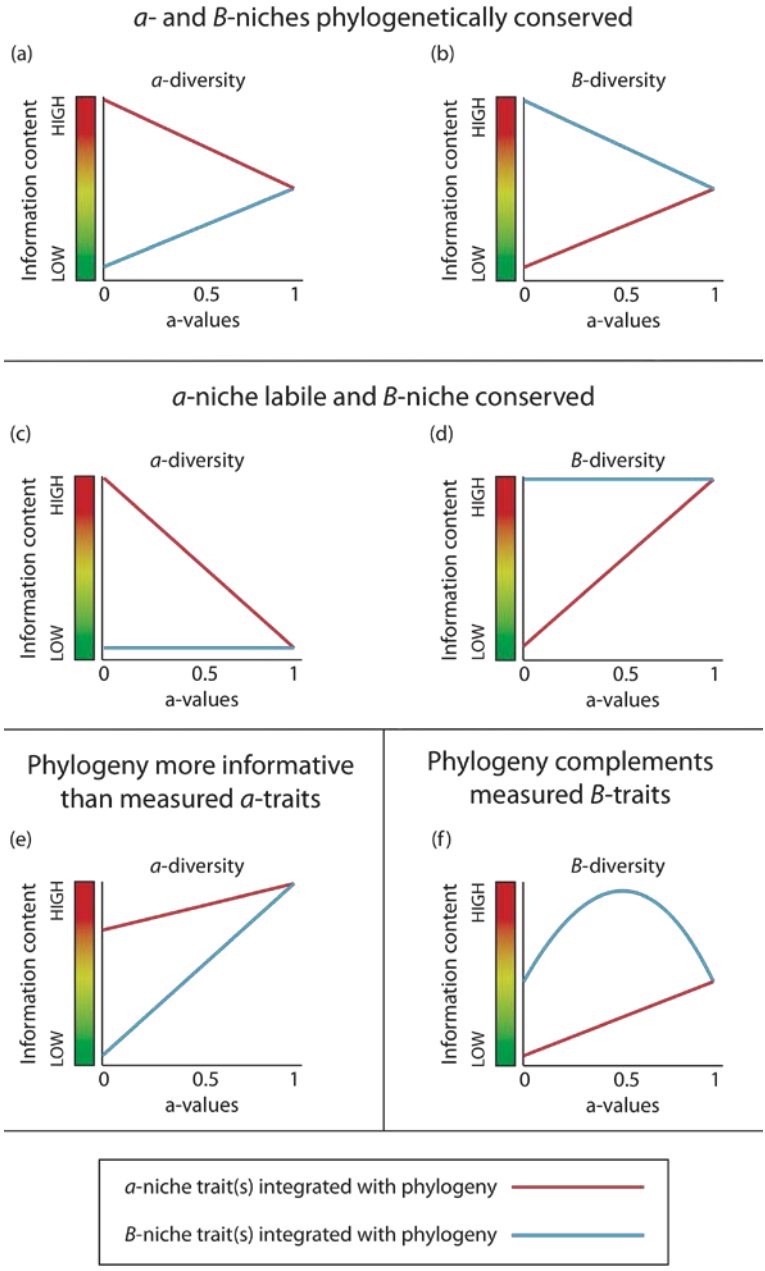

Figure 2. *Conceptual scheme on how to optimally integrate trait and phylogenetic distances in α- and β-diversity analyses. In the vertical axis, information content describes* ***the strength of the response of***

***different traits and phylogeny to α- and β-scale assembly processes**. In the horizontal axis, a-values refer to the phylogenetic weighing parameter, which allows integration of trait and phylogenetic distances [see methods for details and also Cadotte, M. et al. (2013)]. For a-value = 0 only trait information is taken into account; for a-value = 1 only phylogenetic information is considered. Intermediate a-values indicate that both trait and phylogenetic distances are combined. In (a) and (b) both α- and β-niches are phylogenetically conserved so that phylogeny is informative at both spatial scales. In such cases it may be more powerful to account only for α-traits in α-diversity analyses (a) and only for β-traits in β-diversity analyses (b). In (c-d) phylogeny captures information on the β-niche, but not the α-niche, so that phylogeny is uninformative for α-diversity but highly informative for β-diversity analyses (the opposite is true when phylogeny captures information on the α-niche, but not the β-niche; not shown). In (e) phylogeny better represents variation in the α-niche than the measured α-trait(s). The same can occur for β-diversity (not shown). In (f) phylogeny complements the information provided by the measured traits, so that higher information content is obtained for intermediate a-values. This can also happen for α-diversity [not shown; but see also Cadotte, M. et al. (2013)]. The schemes depicted here are a simplification of reality as we assume one single α- and β-niche trait, whereas in reality there are a multitude of traits that are integrated when using phylogenies, and there are a multitude of traits involved in α- and β-niche processes.*

Empirical example with freshwater zooplankton

In the summer of 2003, zooplankton samples were collected in 99 permanent farmland ponds across Belgium (c. 30,500 km^2) (De Bie et al. 2012). To maximize variation in environmental conditions and disturbance levels, a stratified sampling was imposed in which 33 ponds were selected in nature reserves, 33 in extensive agricultural landscapes, and 33 in areas of intensive agriculture. Cladoceran community composition was characterized by counts and individuals were identified to species level (De Bie et al. 2012). We excluded ponds (n=8) where only one species was recorded. Physical, chemical and morphometric pond variables were also collected and are described elsewhere (De Bie et al. 2012) (see also Appendix S1 in supporting information). Based on previous work on cladoceran metacommunities, we selected 12 environmental variables: concentration of total phosphorus and nitrates, pH, water transparency, conductivity, water temperature, submerged vegetation cover, floating vegetation cover, pond area, shaded area of the pond surface, abundance of fish, and presence of the invertebrate predator *Chaoborus*. We also included regional land use as an ordinal variable (i.e. 1 = nature reserve; 2 = extensive agricultural land use; and 3 = intensive agricultural land use). For a detailed description of the sampling methods see Appendix S1.

Trait data

We accounted for two key traits that have been repeatedly suggested to affect zooplankton community structure and turnover: **body size and degree of association with (submerged) vegetation**. Body size is linked to competitive ability and resource partitioning in cladoceran

zooplankton (Brooks and Dodson 1965, DeMott and Kerfoot 1982, Gliwicz and Lampert 1990). Thus, we expected **body size to behave primarily as an *α*-niche trait**, even though it can also be associated with fish predation pressure (Brooks and Dodson 1965) and thus with species turnover across habitats. This illustrates challenges related to trait classification *a priori* in terms of α- and β-niches components. **Species association with (submerged) vegetation is expected to represent an important *β*-trait for cladoceran zooplankton** (Declerck et al. 2007, Declerck et al. 2011). Specifically, depending on their degree of association with submerged vegetation, zooplankton species tend to be sorted out along the typical turbid – clear water gradient in shallow waters (Scheffer 2004). In practice, however, it is likely that the association of traits with *α*- or *β*-niche axes are not mutually exclusive but rather vary in strength.

Information on body size values and on the degree to which the species were associated with (underwater) macrophytes were extracted from the literature. Body size is a continuous variable, whereas the strength of plant association was quantified as an ordinal variable (1 = pelagic; 2 = intermediate (opportunistic, generalist); 3 = littoral, strongly associated with macrophytes). All trait values and associated references can be found in Appendix S1.

Molecular-phylogenetic tree reconstruction

We built a molecular-phylogenetic tree for 35 of the 40 cladoceran species occurring in the sampled metacommunity following a recent protocol (Roquet et al. 2013). Information on four molecular markers (COI, and 16S, 18S and 28S rDNA) was extracted from Genbank for

the 35 species using the browser “Geneious”. Maximum Likelihood (ML) tree reconstruction and bootstrapping was performed using RAxML (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis 2006). Finally, an ultrametric tree was constructed using the Penalized Likelihood method (Kim and Sanderson 2008). A complete description of the method used is given in Appendix S1.

Calculation and integration of phylogenetic and trait distances

A cophenetic phylogenetic distance matrix (PDist) was calculated from the branch lengths of the phylogenetic tree. A Euclidean trait distance matrix ($FDist_{body\ size}$) was calculated using body size values for all zooplankton species in the metacommunity. Finally, we also calculated a Euclidean trait distance matrix based on plant association ($FDist_{plant\ assoc}$).

Using these distance matrices, we constructed functional-phylogenetic distance matrices (FPDist) following Cadotte et al. (2013). We built 101 FPDist matrices corresponding to a values ranging from 0 to 1 at intervals of 0.01. For a = 0, FPDist represents the pure functional trait distances, for a = 1 FPDist only represents the pure phylogenetic distances. Intermediate a-values indicate that both sets of information are integrated. These FPDist matrices were used *a posteriori* to estimate under which relative contribution of functional and phylogenetic information (i.e. at which a-value) our understanding of environmental and spatial drivers on biodiversity patterns is maximized using a variation partitioning approach (see further). We first combined information in our FPDist matrix based on the two traits (body size and strength of plant association), separately.

We then also combined information on body size and plant association with phylogenetic information. Because one trait is categorical and the other is continuous, we used Gower's distance instead of Euclidean distance to calculate the combined trait distance matrix (Borcard et al. 2011).

Phylogenetic signal in traits

We assessed the level of phylogenetic signal in body size and plant association through the K-statistic (Blomberg et al. 2003). The K-statistic can be compared to randomized K values and significant p-values indicate non-random correspondences between traits and phylogeny. Values of K higher than 1 indicate that traits are more conserved than expected by a Brownian Motion Evolutionary model (Blomberg et al. 2003). Values lower than 1 indicate that traits are less conserved along the phylogeny than expected by this model.

Functional-phylogenetic α-diversity across a-values

We calculated the abundance-weighted version of the mean functional-phylogenetic distance across *a*-values (MFPD, hereafter *α-FPD*) within communities. *α-FPD* considers information on the average sum of evolutionary and/or trait distances (depending on the *a*-values) among species in local communities (Cadotte et al. 2013), and quantifies the level of clustering (i.e., communities that contain species that are more similar/more related to each other than expected by chance) or overdispersion (i.e., communities comprised of species that are less similar/less related than expected by chance). In order to ensure that differences in trait-phylogenetic structure were not an

artifact of the number of species, we calculated the standardized effect size. To do this, we shuffled species names in the distance matrix 999 times to create a functional-phylogenetic null distribution of taxa in local communities, keeping the entire community data matrix constant (i.e. abundance values and frequencies remained untouched) (Swenson 2014).

Functional-phylogenetic β-diversity across a-values

β-diversity was calculated based on each of the 101 FPDist matrices using mean pairwise functional-phylogenetic dissimilarity among pairs of local communities (Swenson 2014), using the function COMDIST in picante statistical package (Kembel et al. 2010). For simplicity, we hereafter refer to this index as *β*-FPD. Abundance values of species were considered when calculating *β*-FPD. We then applied Principal Coordinates Analysis (PCoA) over the 101 *β*-FPD dissimilarity matrices (Swenson 2014). The final product is a matrix of orthogonal PCoA eigenvectors, each of them describing functional-phylogenetic *β*-diversity patterns, which can be used as response variables in constrained ordinations (Anderson and Willis 2003, Swenson 2014).

Taxonomic α- and β-diversity

We used traditional taxon-based community analyses as a benchmark to assess the performance gain achieved by more ecologically detailed trait- and phylogenetic approaches. To describe taxonomic *α*- and *β*-diversity patterns we choose widely used indices that take into account abundance values of species, making it comparable to the above-

described functional-phylogenetic indices. We used the exponent of the Shannon-Wiener index (i.e., Shannon entropy, Jost 2006) as our metric of taxonomic α-diversity patterns. We compared results obtained for Shannon entropy with those obtained for taxonomic species richness and evenness (see Appendix S1). Results obtained for these three taxonomic diversity metrics were very similar (Appendix S1), and therefore we report in the main text only results of Shannon entropy. As a metric of taxonomic β-diversity, we used the Bray-Curtis index of dissimilarity. We then applied PCoA to the Bray-Curtis dissimilarity matrix using Lingoes correction (Borcard et al. 2011) and kept the eigenvectors to be used as response variables in the subsequent analyses.

Generating spatial descriptors

We used the geographical coordinates (UTM) of the sites to generate spatial variables by means of Principal Coordinates of Neighboring Matrices (PCNM) (Borcard and Legendre 2002). This technique allows assessing multiple spatial structures over the entire range of scales covered by the geographical sampling area. The first PCNMs generated in the analyses represent broader spatial structures, while the last ones cover finer spatial scales (Borcard and Legendre 2002). We retained for subsequent analyses only significant PCNMs associated with positive eigenvalues (n = 18).

Selection of response variables: orthogonal eigenvectors describing β-diversity patterns

In principle, all eigenvectors generated by PCoA could be used as descriptors of β-diversity patterns in subsequent analyses. However, using all of them might introduce confounding effects in the analyses. Since each eigenvector is an orthogonal synthetic variable representing gradients in β-diversity patterns, it is likely that some of these gradients are unexplained by the measured factors. A solution is to select a subset of orthogonal eigenvectors that maximizes the association between patterns of β-diversity (taxonomic or functional-phylogenetic) and a set of explanatory variables. To select the appropriate number of PCoA eigenvectors to be used in subsequent analyses, we followed the procedure proposed by Anderson and Willis (2003) (for more details see Appendix S1).

Disentangling the environmental and spatial drivers of α- and β-diversity

Variation partitioning allows disentangling the variation in community data (here also weighed by trait-phylogenetic distances) into a pure environmental component, a pure spatial component, a spatially structured environmental component, and an unexplained component of variation (Borcard et al. 1992). Before performing variation partitioning, explanatory variables were selected using forward selection (Blanchet et al. 2008). We selected subsets of spatial and environmental variables for each *FPD*-metric (i.e., across *a*-values). This allowed us to maximize the fit between explanatory and response data matrices while minimizing type I error. We also compared these results with analyses keeping the same number and type of explanatory variables for the entire range of *a*-values. Results of both

approaches were qualitatively very similar, thus we report the results of the former approach only, which has a better compromise between explanatory power and type I error. Following Cadotte et al. (2013) we used the full model $_{adj}R^2$ (i.e., environment and space combined) as our measurement of information content for different combinations of trait and phylogenetic information (i.e., across *a*-values) for α- and β-diversity analyses. Based on the conceptual scheme in figure 2, we tested the idea that functional diversity is structured by different traits at different scales. This idea is supported when the most informative trait at one spatial scale (i.e., quantified by the $_{adj}R^2$; *a*-value = 0) is the least informative at the other spatial scale and vice-versa (Fig. 2). Similarly, we tested the idea that combining α- and β-traits reduces overall explanatory power (Fig. 1). This was done by quantifying the decrease in informative power ($_{adj}R^2$) obtained for single traits and that obtained when combining both traits in α- and β-diversity analyses. To test the idea that phylogenetic distances are informative at both spatial scales but can be outperformed by trait-based analyses that ensure a good match between trait and spatial scale, we quantified, for each spatial scale the increase or decrease in information content ($_{adj}R^2$) obtained by increasing phylogenetic weighing (i.e., increasing *a*-values; Fig. 2). All analyses were performed in R (R Core Team 2014) and a detailed description of the methods and packages used can be found in Appendix S1.

RESULTS

Taxonomic patterns for α- and β-diversity

Taxonomic α-diversity was not significantly explained by any environmental or spatial variable (Table 1). For the taxonomic β-diversity, environmental and spatial factors combined explained approximately 18% of variation in species composition among ponds ($_{adj}R^2$ = 0.182, p = 0.001). From this total, about three quarters was determined purely by the environmental model (i.e, submerged vegetation; *Chaoborus*; pH and nitrates), approximately one quarter was purely explained by spatial processes, and the shared effects of spatial and environmental variables accounted for 1.5% (Table 1).

Table 1. *Proportion ($_{adj}R^2$) of the total variation in α-FPD and β-FPD explained by the total environmental and spatial model (EUS), by a pure environmental signal (E/S), by a pure spatial signal (S/E), and by the shared effect of space and environment (E∩S), as estimated by variation partitioning, and the corresponding p-values. For trait-phylogenetic metrics we only report the best combination between trait and phylogenetic information and list the a-value for which this maximum is obtained. Body size + Phylogeny refers to diversity metrics integrating information on body size and phylogeny. Plant association + Phylogeny refers to diversity metrics integrating information on plant association and phylogeny. Body size and plant association + Phylogeny refers to diversity metrics integrating information on both traits with phylogeny.*

Index	**$_{adj}R^2$**			
	EUS	**E/S**	**S/E**	**E∩S**
α-diversity				
Taxonomic (Shannon	0	0	0	0

Index	$_{adj}R^2$			
	EUS	**E/S**	**S/E**	**E∩S**
entropy)				
Body size + Phylogeny				
α-FPD (*a* = 0) (i.e., pure body size)	0.228***	0.091**	0.074**	0.061
α-FPD (*a* = 0.01) (maximal R^2 obtained)	0.229***	0.092**	0.071**	0.064
Plant association + Phylogeny				
α-FPD (*a* = 0) (i.e., pure plant association)	0	0	0	0
α-FPD (*a* = 0.73) (maximal R^2 obtained)	0.131***	0.131***	0	0
α-FPD (*a* = 1) (i.e., pure phylogeny)	0.060**	0.060**	0	0
Body size and plant association + Phylogeny				
α-FPD (*a* = 0) (maximal R^2 obtained)	0.141***	0.141***	0	0
β-diversity				
Taxonomic (Bray-Curtis)	0.182***	0.121***	0.045**	0.015
Body size + Phylogeny				
β-FPD (*a* = 0) (i.e., pure body size)	0.175***	0.175***	0	0
β-FPD (*a* = 0.71) (maximal R^2 obtained)	0.296***	0.296***	0	0

Index	$_{adj}R^2$			
	EUS	**E/S**	**S/E**	**E∩S**
Plant association + Phylogeny				
β-FPD (*a* = 0) (i.e., pure plant assoc.)	0.483***	0.483***	0	0
β-FPD (*a* = 0.04) (maximal R^2 obtained)	0.495***	0.495***	0	0
β-FPD (*a* = 1) (i.e., pure phylogeny)	0.291***	0.291***	0	0
Body size and plant association + Phylogeny				
β-FPD (*a* = 0.13) (maximal R^2 obtained)	0.410***	0.410***	0	0[1]

Phylogenetic signal in traits

K–statistics revealed that body size was not significantly conserved along the phylogeny (K = 0.04, *p* = 0.243). In contrast, plant association was strongly conserved along the phylogeny (K = 2.034, *p* = 0.001).

[1] Significant results are indicated with asterisks as follows: $p < 0.001$ "***"; $p < 0.005$ "**".

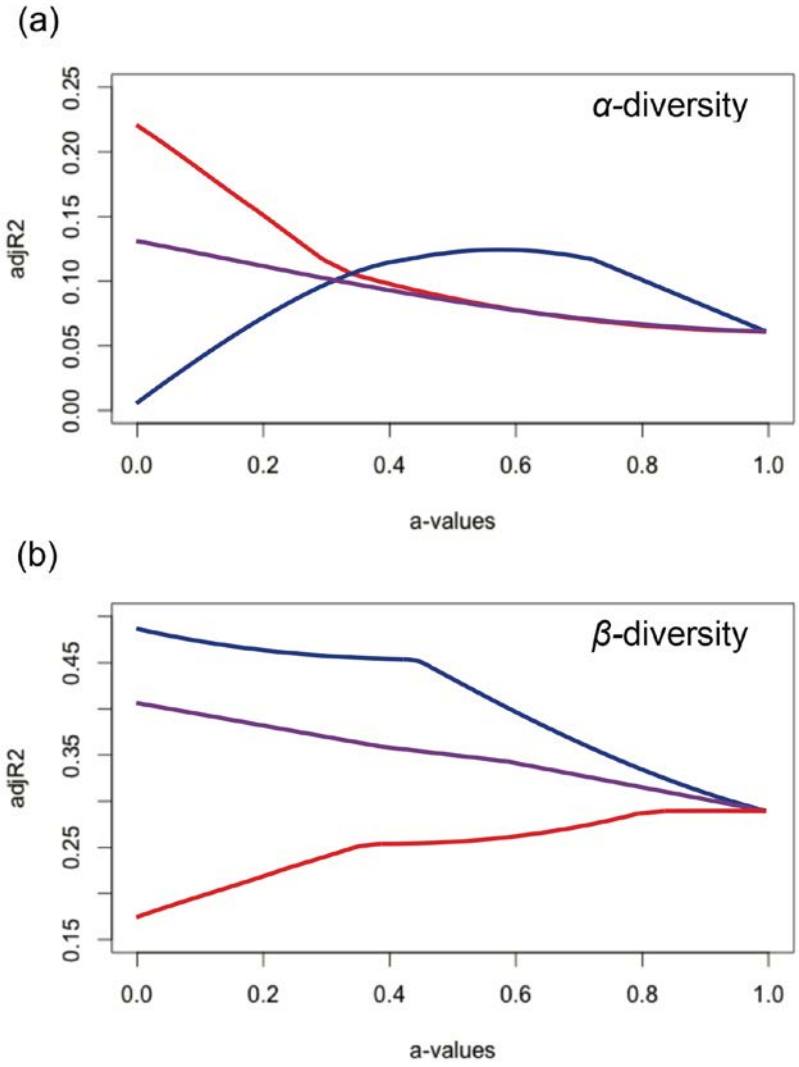

Figure 3. *Proportion of total explained variation (i.e., environmental and spatial processes combined; as given by* $_{adj}R^2$*) for functional-phylogenetic α-diversity (a) and β-diversity (b) across a-values (a ranges from 0 where only trait information is taken into account to 1 where only phylogenetic information is considered, in steps of 0.01). At intermediary a values, both trait distance and phylogenetic*

distance are combined. The red line refers to analyses where body size information is combined with phylogenetic information; the blue line refers to analyses where plant association information is combined with phylogenetic information. The purple line refers to a situation in which both traits are combined with phylogenetic information. LOESS smoothing was used to aid visualizing and interpreting the general patterns.

Testing idea I: Is functional diversity structured by different traits at different scales?

When analyzing traits (a = 0) separately in α-diversity analyses we found that body size responded stronger than plant association to both environmental and spatial processes operating at this local scale (Fig. 3a, Table 1). Body size was 22.8% more informative (as measured by differences in $_{adj}R^2$) than plant association for α-diversity analyses. Conversely, β-diversity analyses indicated that plant association responded much stronger than body size to (environmental) processes, even though body size was still informative for β-scale analyses (Fig. 3b, Table 1). Plant association was 30.8% more informative than body size for β-*d*iversity analyses. Environmental (pH and NO^3) and spatial variables were similarly important in affecting body size diversity at local scales (Table 1). With respect to the environmental signal at the local scale, we observed a shift from size clustering to overdispersion along a gradient of pH (Appendix S2). Importantly, the pattern of size overdispersion in ponds with high pH was accompanied by an increase in abundance of larger species (Fig. 4). Under low pH values, small species dominated, while at higher pH values both small- and large-bodied species coexisted (see Fig. S2 in Appendix S3).

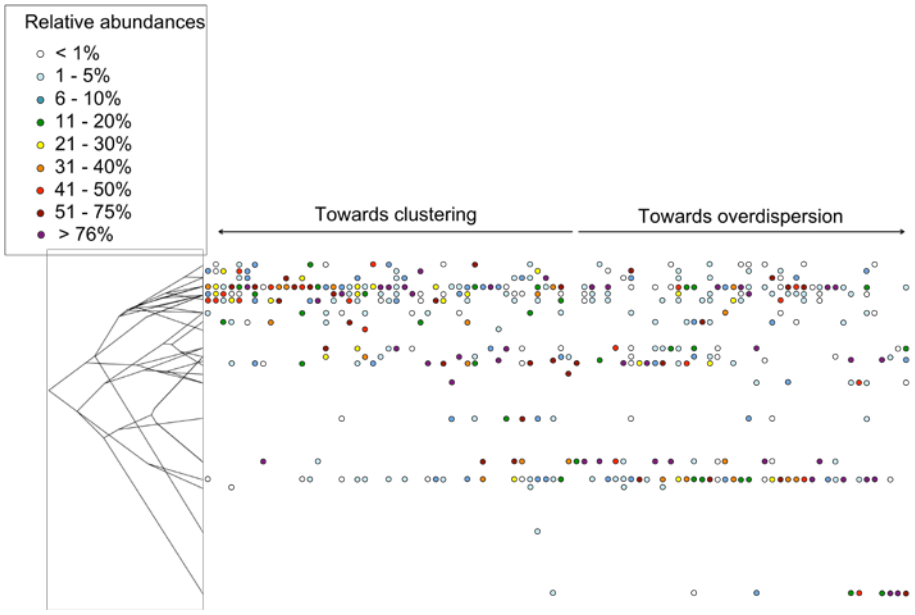

Figure 4. *Evolutionary-trait gram depicting patterns of trait and phylogenetic (i.e., α-FPD body size + phylogeny) clustering versus overdispersion for each of the communities of all the 91 sampled ponds. The evolutionary-traitgram combines phylogenetic and trait information and is useful to assess patterns of trait evolution (i.e., divergences, convergences) because it posits the tips of the phylogeny according to a trait axis (here body size, ranging from 0.26 on top of the vertical axis to 4 mm on the bottom), while keeping the internal nodes proportional to evolutionary time (i.e., genetic distance in this example). Each column of circles represent one of the 91 sampled ponds and the color of each of the circles represents the relative abundance of each species represented at the tip of the phylogeny in each of the ponds. Communities are ordered according to α-FPD, ranging from clustered to overdispersion.*

Testing idea II: Combining traits reduces overall explanatory power

We found that combining both body size and plant association in trait-based analyses (a = 0) was suboptimal compared to accounting separately for body size at the α-scale and for plant association at the β-scale (Fig. 3, Table 1). In contrast, we found that integrated trait indices outperformed indices accounting solely for body size alone when this trait was used at the β-scale (Fig. 3b). Integrated trait indices similarly outperformed an index weighed by plant association alone for α-diversity analyses (Fig. 3a, Table 1).

Testing idea III: Phylogeny is informative at both spatial scales but is outperformed by trait analyses accounting only for the most informative trait at each spatial sale

Integrating trait and phylogenetic information via phylogenetic weighing revealed that the explanatory power of α-diversity patterns was maximized when focusing on body size information and decreased as more phylogenetic information was considered (i.e., towards large *a*-values) (Fig. 3a, Table 1). This indicates that a single trait (body size) was more informative than phylogeny in determining diversity patterns at this spatial scale. Conversely, integrating information on plant association and phylogeny resulted in increasing explanatory power with increasing phylogenetic weighting (i.e., towards large *a*-values) for the analysis of α-diversity (Fig. 3a, Table 1). For a complete overview of ${}_{adj}R^2$ values and the list of significant explanatory variables across *a*-values see Appendix S2.

When considering β-diversity patterns, we observed that the maximal explanatory power was obtained towards pure plant association information, and decreased with increasing phylogenetic weighing (towards large *a*-values) (Fig. 3b, Table 1). In contrast, while the correlation between explanatory variables and body size was significant (Table 1, Appendix S2), accounting for body size information was less informative than accounting for phylogenetic information at this spatial scale (Fig 3b).

DISCUSSION

Increasingly, trait and phylogenetic data is being used to get new insights into biodiversity drivers across spatial scales. The underlying assumption is that using phylogenetic data or combining different traits into trait-based analyses can capture a significant portion of species fundamental niches, thus enhancing our understanding of the processes structuring biodiversity (Mouquet et al. 2012, Spasojevic et al. 2014). Here we examined community assembly in freshwater zooplankton and found that mixing traits associated with processes that operate at different spatial scales (here: α- and β-scale processes) in diversity metrics was suboptimal compared to using a single, most informative trait for each spatial scale. This supports that idea that the measured traits are associated with different axes of niche space and can provide complementary insights on community assembly across spatial scales. The most sensitive trait to α-scale processes, body size, contributed little to diversity patterns among communities, while plant association was very strongly related with species turnover among habitats but was not informative for α-diversity. At both scales, using

the most informative trait resulted in a higher explanatory power of environmental and/or spatial processes than incorporating phylogenetic distances. However, phylogenetic distances were more informative than the same traits when these were used at the wrong spatial scale, suggesting that phylogeny represents both α- and β-niches (Fig. 2a-b). Integrating phylogenetic and trait information is thus important in adding a benchmark to scale the explanatory value of specific traits, and allows optimization of explanatory power in terms of unmeasured, phylogenetically conserved traits. Turning this reasoning around, it is also possible, by measuring phylogenetic signal in traits associated with processes at different spatial scales (Blomberg et al. 2003), to understand how long-term evolutionary processes affect current species distributions across spatial scales.

Body size was more informative for α-diversity patterns than phylogeny, plant association or taxonomic information. Surprisingly, fish abundance – often a key variable determining zooplankton size structure (Brooks and Dodson 1965) - was not significantly related with body size α-diversity in our study. A potential explanation for this is that most of the ponds in our regional metacommunity were either fishless or presented low fish abundances. Conversely, we observed that local communities shifted from clustering to overdispersion along a gradient of pH. In our study system, pH ranged from approximately 6.5 to 9.5 (Appendix S3), so that the pattern of trait clustering in ponds with low pH unlikely results from physiological stress associated with acidic conditions, as reported for Canadian lakes (Helmus et al. 2010). While it is not possible to identify the mechanism mediating the positive relationship between

size diversity and pH from our dataset with any certainty, it may reflect a response to a productivity gradient. High pH values in aquatic systems are often associated with high productivity [alkaline lakes being often more productive, and the consumption of CO_2 by photosynthesis increasing pH directly; (Moss 2010)], which in turn tends to favor dominance by the larger *Daphnia* species (Dodson et al. 2000). To further explore this possibility, we quantified to what extent pH in our dataset was correlated to environmental variables linked to productivity. We indeed observed an overall positive correlation between values of pH, turbidity, total phosphorus, and chlorophyll *a* concentrations, which are all productivity related variables (total phosphorus being the key limiting nutrient in most freshwater systems and chlorophyll a quantifying algal biomass, see Appendix S3 for the relationships). For cladoceran zooplankton, observational and experimental studies have demonstrated that low productivity may result in the elimination of larger species from communities likely due to food limitation (Tillmann and Lampert 1984, Brett et al. 2000, Dodson et al. 2000). Low pH or low associated productivity may thus have acted as a filter on body sizes in our study systems, preventing the establishment success of large species (Appendix 3). Conversely, the establishment of larger species in more productive ponds with higher pH may have resulted in the observed pattern of overdispersion in these ponds. We also note that the presence of the invertebrate predator *Chaoborus*, along with pH, significantly explained the shift from small to large zooplankton species in our system of ponds (Appendix S2, Table 5). This predator tends to select smaller zooplankton species (Riessen et al. 1988), so that predator release may

have allowed small species to dominate in some ponds, resulting in size clustering.

While body size was very informative and plant association was uninformative for α-diversity, the information content of phylogenetic information was intermediate. The decrease in explanatory power from pure body size towards phylogenetic information within communities probably reflects the lack of phylogenetic signal in body size and suggests that size variation is more structured by the studied environmental and spatial drivers than other, more conserved traits. Lack of phylogenetic signal in key traits is known to erode the phylogenetic signal on community assembly (Mouquet et al. 2012, Wiens et al. 2010). Similar results were found for butterflies (Pavoine et al. 2014) and marine herbivores (Best et al. 2013), in which lack of conservatism in key traits associated with species coexistences resulted in low phylogenetic signal on α-diversity.

Plant association had high explanatory power for β-diversity in the zooplankton metacommunity we studied, reflecting strong habitat filtering. Specifically, we observed that as environmental conditions changed along the typical turbid – clearwater habitat gradient in shallow waters (Scheffer 2004), zooplankton communities shifted from a dominance of pelagic species to a dominance of littoral species. This resulted in a pattern of clustering within communities and a high turnover among communities. The patterns for α- and β-diversity observed for plant association are therefore strongly linked, as they both reflect habitat filtering [see also Messier et al. (2010) for a similar example]. Similarly, for body size the patterns of α- and β-

diversity are also linked, as the strong signal for α-diversity is the result of a shift from clustering to overdispersion, which is fueled by species turnover involving large species being excluded from low pH systems. Although body size is less informative for β-diversity than plant association or phylogenetic information, it still explains 17% of the observed variation in communities among habitats.

Despite the strong phylogenetic signal in plant association, explanatory power for β-diversity analyses decreased from pure plant association towards pure phylogenetic information. While this seems counterintuitive at first sight, it likely reflects the fact that incorporating information on multiple trait axes that are associated with different processes may reduce the explanatory power of phylogenetic analyses (Butterfield and Suding 2013). Indeed, a recent simulation study demonstrated that such contrasting signals among phylogenetically conserved traits affect the performance of phylogenetic metrics (Trisos et al. 2014). Recent empirical studies have similarly shown that different traits can respond in contradictory ways to environmental gradients, so that focusing on single traits in separation may increase the explanatory power and interpretability of diversity patterns compared to multi-trait and phylogenetic analyses (Butterfield and Suding 2013, Spasojevic and Suding 2012). This is also illustrated by our own trait-based analysis, where we observed that including body size and plant association was suboptimal compared to using the best single trait for each scale of species distributions.

While our results show the usefulness of distinguishing traits for their association with within- or among-habitat processes, they

also highlight that patterns in nature are more complex and that traits can behave both as α- and β-niche traits. For example, we observed that cladoceran body size was very important for α-diversity, but at the same time was still important for β-diversity patterns, as the pH gradient and the presence of the predator *Chaoborus* created a shift in body size among ponds. The degree to which a trait may be more α- or β-niche related can vary among metacommunities and environmental conditions. It is therefore not an easy task to identify *a priori* which trait should be classified as α- and β-niche traits. Instead, the method we apply here can actually be used as a diagnostic tool to determine the degree to which a trait is associated with α- and β-niches, using phylogenetic information as a benchmark (Fig. 2). This is important because, due to limitations in time and resources, trait-based approaches often depend on *a priori* decisions on which traits should be measured. In our data set, phylogenetic distances did provide additional explanatory power at both spatial scales over a non-informed taxonomic analysis based on species identity alone and also when there was a trait-scale mismatch, such as when using the β-niche trait to study α-diversity patterns. Our approach differs from that proposed by Ackerly and Cornwell (2007) in that it does not rely exclusively on intraspecific trait information. Our approach integrates trait and phylogeny to account for potentially informative, unmeasured α- and β-traits, and it can be used in a multivariate environmental and spatial context.

Besides increasing the overall explanatory power of the analyses, the trait-phylogenetic approach also differed in the relative importance of environmental and spatial drivers of metacommunity

structure compared to the taxonomic approach. The variation partitioning analysis indicated that taxonomic β-diversity was both structured by environmental determinism as well as pure spatial processes. The influence of spatial processes was, however, not significant when we analyzed trait and phylogenetic β-diversity patterns. We see at least two plausible explanations for this. First, as we include more trait-related information, we better approximate species niches, so that we better capture the impact of ecological determinism in structuring communities. Alternatively, the pure spatial signal for taxonomic β-diversity may reflect true dispersal limitation, which is not occurring at the trait or phylogenetic level. This would occur when, for example, communities are assembled through trait sorting, while there is dispersal limitation with respect to which species represents a given trait. The lack of spatial constraints on the phylogenetic species pool is in line with previous findings for freshwater cladoceran communities in North America (Leibold et al. 2010). While we did not observe a pure spatial signal in the trait-phylogenetic analysis of β-diversity, body size α-diversity was spatially structured. To explain this, we conducted additional analyses, which reveal that this pattern is likely mediated by dispersal limitation of large-bodied species, which are strong competitors (see Appendix 4). Hence, traits related to α- and β-scale processes also differed in their environmental determinants and in the importance of spatial signals. This difference was not detectable when considering overall functional diversity. Thus, accounting separately for traits related to different environmental gradients and spatial scales allows more

straightforward interpretation of the drivers of diversity within and among communities.

In summary, our results support the hypothesis that different functional traits can be associated with distinct aspects of species' niche use, and that confounding effects among such traits compromise the performance of metacommunity analysis compared to single trait metrics (Butterfield and Suding 2013, Spasojevic and Suding 2012). Such confounding effects can also affect the performance of phylogenetic metrics (Trisos et al. 2014). To overcome these limitations, we propose an approach in which α- and β-traits are analyzed separately and evaluated for their explanatory power using phylogenetic information as a benchmark in an integrated analysis of functional and phylogenetic distances (Fig. 2). We demonstrated that phylogenetically informed analysis provides a strong benchmark for assessing the explanatory power of trait-based approaches at different spatial scales. Therefore, if measured traits do not match the spatial scales considered or if there is lack of trait data, phylogeny provides a valuable substitute for taxonomy-based community approaches. While we here only considered local and regional spatial scales, we envision that a similar approach can be used to analyze patterns ranging from local to continental and global scales.

ACKNOWLEDGMENTS:

We thank Jason Pither, Jelena Pantel, Matthias Vanhamel and several anonymous referees for valuable suggestions on early versions of the manuscript. ATG received a full PhD scholarship from Brazil-CNPq 245629/2012-2 under the program "Science without Borders". This

study was financially supported by the KU Leuven Research Fund Centre of Excellence funding PF/2010/07, Belspo IAP project SPEEDY (P7-04) and Belspo-funded ERA-Net BiodivERsA project TIPPINGPOND.

SUPPLEMENTARY INFORMATION

Appendix S1. Detailed description of methods

Appendix S2. Supporting statistics

Appendix S3. Productivity gradient and its relation with body size diversity

Appendix S4. Spatial signal on body size diversity

CHAPTER IV

DISENTANGLING THE EFFECT OF BODY SIZE AND PHYLOGENETIC DISTANCES ON ZOOPLANKTON TOP-DOWN CONTROL OF ALGAE

[Modified from Proceedings of the Royal Society B: Biological Sciences (2016), doi: 10.1098/rspb.2016.0487]

Andros T. Gianuca, Jelena H. Pantel and Luc De Meester

ABSTRACT

A negative consequence of biodiversity loss is reduced rates of ecosystem functions. Phylogenetic-based biodiversity indices have been claimed to provide more accurate predictions of ecosystem functioning than species diversity alone. This approach assumes that the most relevant traits for ecosystem functioning present phylogenetic signal. Yet, traits mediating niche partitioning and resource uptake efficiency in animals can be labile. To assess the relative power of a key trait (body size) and phylogeny to predict zooplankton top-down control on phytoplankton, we manipulated trait and phylogenetic distances independently in microcosms while holding species richness constant. We found that body size provided strong predictions of top-down control. In contrast, phylogeny was a poor predictor of grazing rates. Size-related grazing efficiency asymmetry was mechanistically more important than niche differences in mediating ecosystem function in our experimental settings. Our study demonstrates a strong link between a single functional trait (i.e. body size) in zooplankton, and trophic interactions, and urges for a cautionary use of phylogenetic information and taxonomic diversity as substitutes for trait information to predict and understand ecosystem functions.

BACKGROUND

The impact of humans on earth has strongly increased in recent decades, leading to accelerated rates of species extinctions (Pimm et al. 2014). One important consequence of species losses is reduced rates of ecosystem processes, which can be detrimental for ecosystem functioning and the provisioning of ecosystem services (Cardinale et al. 2012, Naeem et al. 2012, Lefcheck et al. 2015). Several studies manipulating the number of species within communities have indeed demonstrated a positive relationship between biodiversity and ecosystem functioning (Cardinale et al. 2006, Cardinale et al. 2012, Lefcheck et al. 2015). However, ecosystem functioning can vary when richness is held constant (Cardinale et al. 2006). This variance likely results from niche and evolutionary differences among species (Srivastava et al. 2012b). Biodiversity indices that integrate species ecological similarities and differences should therefore improve predictions of ecosystem functioning (Srivastava et al. 2012b, Cadotte 2013).

Species often differ in their resource use and may also differ in resource uptake efficiency (Carroll et al. 2011). Because of such functional differences, the local extinction of certain species from an ecosystem can have a greater impact on ecosystem functioning than the extinction of other species (Larsen et al. 2005, Winfree et al. 2015). Correctly identifying and accounting for relevant species differences in biodiversity measurements is thus a key step towards more predictive biodiversity ecosystem-functioning (BEF) research. Functional diversity indices incorporate information on one or more

key functional traits and thus provide a single metric to quantify ecological similarities and differences among species, which can help elucidate the mechanisms underlying BEF (Hillebrand and Matthiessen 2009). However, it is often difficult to ascertain that the most relevant traits were included or to measure all relevant traits for multiple species. Phylogenetic distance has been suggested as an alternative way to account for overall species similarities and differences (Srivastava et al. 2012b). This approach assumes that relevant traits for ecosystem functioning are conserved along the phylogeny, with more closely related species resembling each other more in trait values than distantly related species (Wiens et al. 2010). A number of recent studies have indeed provided evidence that evolutionary diversification could lead to more diversification in traits, and thus enhance ecosystem functioning (Gravel et al. 2012, Cadotte 2013). Nevertheless, traits mediating resource use in animals can be labile, which might obscure the relationship between phylogenetic diversity and the functioning of ecosystems, leading to inaccurate predictions (Srivastava et al. 2012b, Best et al. 2013, Cadotte et al. 2013). Consequently, the relative predictive power of traits and phylogenies on ecosystem functioning will ultimately depend on which traits are measured and which traits are phylogenetically conserved or labile.

There are several ways by which trait and phylogenetic information might vary in their relative importance for explaining variation in ecosystem functioning. First, if all relevant traits for the ecosystem function are conserved along the phylogeny and were properly accounted for in a trait-based analysis, phylogenetic and trait-

based indices should provide similar and strong explanatory power. Yet, to the extent that traits are labile, directly accounting for trait information might provide more explanatory power than phylogenetic information. If additional traits that are relevant to the ecosystem function are phylogenetically conserved, then phylogenetic information may still add to the explanatory power provided by the measured (labile) traits, such that a combined analysis of trait and phylogenetic information would be ideal (Flynn et al. 2011). Finally, if none of the traits relevant to ecosystem functioning are phylogenetically conserved, phylogenetic information will have no explanatory power for the studied ecosystem function (Wiens et al. 2010). An experimental system that lacks conservatism in a key trait relevant to ecosystem function presents an ideal setting to test whether phylogenetic distances can represent variation in additional ecological information relevant for ecosystem functioning. In such cases, *a priori* selected traits and unmeasured, evolutionarily conserved traits could complementarily explain ecosystem functioning (Cadotte et al. 2008, Flynn et al. 2011, Cadotte et al. 2013).

Most studies assessing the role of phylogenetic diversity on ecosystem functioning have focused on a single trophic level, primary producers (Flynn et al. 2011, Cadotte 2013), thus hindering generalizations. Given the relevance of trophic interactions for ecosystem functioning (Persson et al. 2007), it is an important next step to test the capacity for phylogenetic distances to predict ecosystem functions at higher trophic levels. In freshwater ecosystems, herbivorous zooplankton grazing plays an important functional role by controlling algal blooms and transferring energy

and matter upwards through the food chain (Persson et al. 2007). Body size of zooplankton has been repeatedly suggested to play a key role in top-down control of algae (Mourelatos and Lacroix 1990, Lampert and Sommer 2007), and this trait was found to be evolutionarily labile in cladoceran zooplankton (see electronic supplementary material for details). The importance of body size for top-down control is generally attributed to grazing efficiency asymmetry (selection effect), where larger species are superior grazers (Fig. 1a) (Brooks and Dodson 1965, Mourelatos and Lacroix 1990) and therefore increasing community average size might enhance grazing rates (Fig. 1c) (Ye et al. 2013). Alternatively, it has been suggested that feeding niche partitioning (complementarity) between large and small zooplankton species may (co)determine top-down control on phytoplankton (Fig. 1b). This latter hypothesis is based on the assumption that larger zooplankton species would be more efficient grazers on larger phytoplankton while small grazers would deplete small phytoplankton more efficiently (Fig. 1b) (Hansen et al. 1994, Cyr and Curtis 1999, Ye et al. 2013). In such a case of size related diet partitioning (complementarity) it is expected that body size diversity, not community average size, would better explain variation in grazing rates among systems (Fig. 1c).

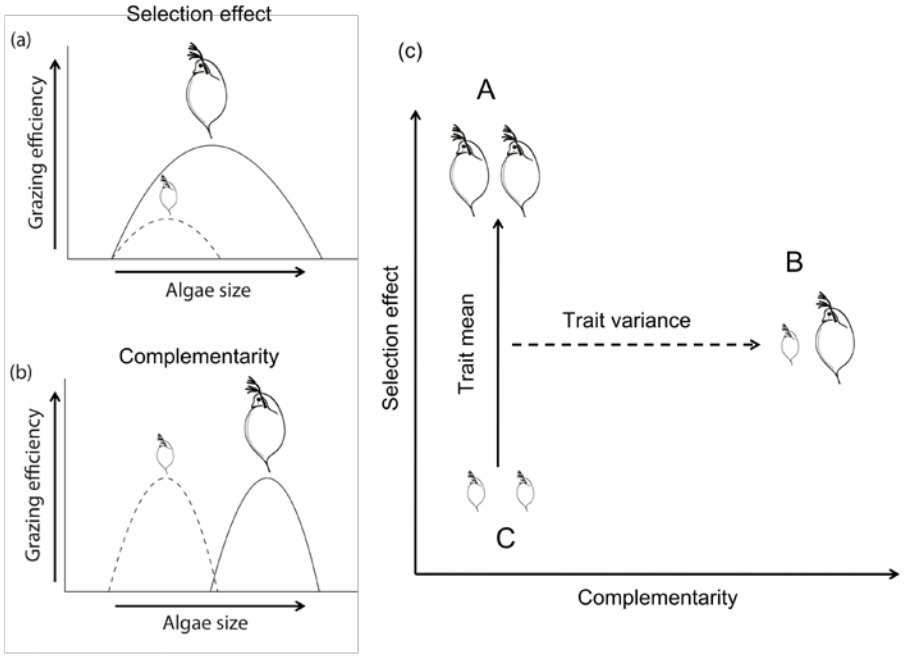

Figure 1. *Schematic representation of alternative mechanisms of how community trait mean and variance can impact ecosystem functioning through selection effects and complementarity. (a) Selection effects (grazing efficiency asymmetry in our system) happen when dominant trait values in a community enhance ecosystem functioning. For instance, in freshwater systems zooplankton species may strongly overlap in resource use but larger zooplankton species are often superior grazers (Mourelatos and Lacroix 1990). (b) Size-mediated complementarity happens when large and small zooplankton species explore alternative resources [e.g., large versus small phytoplankton (Cyr and Curtis 1999, Ye et al. 2013)]. (c) Selection effects and complementarity result in differences in the expected relationship between community trait mean and variance and ecosystem functioning. Three hypothetical communities are represented as A, B and C. Increasing average body size is expected to affect ecosystem*

functioning when grazing efficiency asymmetry (selection effect) is the dominant mechanism that explains differences in ecosystem functioning. This may happen independently of size diversity (i.e., from community C to A, marked by solid line). Alternatively, increasing size diversity (trait variance) within communities may enhance ecosystem functioning when complementarity is important (dashed line) (Ye et al. 2013).

While the relationship between zooplankton body size and grazing rates has been previously documented (Mourelatos and Lacroix 1990, Ye et al. 2013), the role of zooplankton phylogenetic diversity in this context has been overlooked, mainly because most previous studies focused on species of the genus *Daphnia*. We here broadened the taxonomic scope to test whether phylogenetic diversity provides a more comprehensive and accurate representation of species similarities and differences over multiple niche axes than body size alone, complements the information obtained from body size, or does not add any relevant information to predict grazing pressure. A strong signal of zooplankton phylogeny on top-down control would indicate evolutionary conservatism in additional, less explored functional traits that impact grazing efficiency in this system (Thompson et al. 2015).

In order to assess the relative power of functional (body size) and phylogenetic distances to predict top-down control of cladoceran zooplankton on unicellular algae, we designed a microcosm grazing experiment in which zooplankton species pairs varied independently in body size and evolutionary relationships. We combined species based on their trait and phylogenetic distances in order to reflect body

size convergence (i.e., distantly related species overlapping in body size), body size conservatism (i.e., proportional trait and phylogenetic distances), or body size divergence (Fig. 2). Specifically, we tested three key hypotheses: (i) Phylogenetic distances better approximate species similarities and differences and thus provide more accurate predictions of top-down control than accounting solely for body size. (ii) Body size and phylogeny provide complementary information on top-down control, thus higher predictive power is achieved when both body size and phylogenetic information is taken into account. (iii) Zooplankton body size is the most relevant trait and better predicts top-down control than phylogeny, and therefore phylogenetic information does not contribute to predict grazing pressure. For each species pair we measured grazing rates under standardized conditions. By comparing these community grazing rates with those of all species in monoculture, we could assess to what extent the observed patterns were explained by grazing efficiency asymmetry (selection effect) or by niche differences (complementarity) among species (Fig. 1a-b). If selection effects (Fig. 1a) represent the dominant mechanism driving top-down control, we expect that community average size will better predict top-down control than size diversity (Fig. 1c). Conversely, if size-related complementarity is the prevalent mechanism (Fig. 1b), we expect that differences in size diversity will better predict top-down control (Fig. 1c).

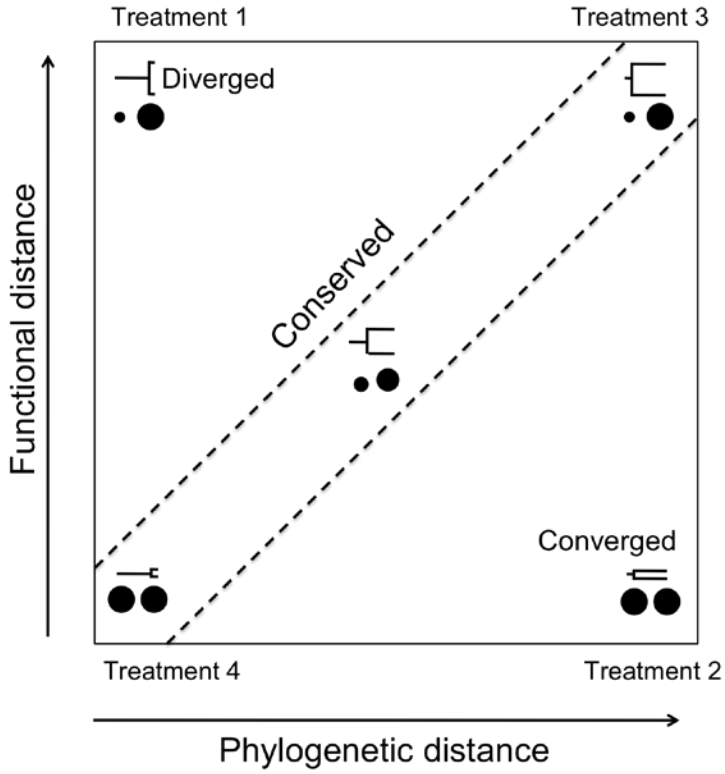

Figure 2. *Scheme depicting the different treatments creating combinations of species pairs that differ in body size and / or phylogenetic distances. Treatment (i) represents closely related species that have diverged in size; treatment (ii) refers to distantly related species that have converged in size; treatment (iii) represents a situation of trait conservatism along the phylogeny, in which distantly related species are dissimilar in size; treatment (iv) refers to closely related species that are similar in body size. Adapted from Cadotte et al. (2013).*

METHODS

Experimental organisms and trait/phylogenetic diversity treatments

We selected species pairs from a pool of eight freshwater Cladoceran species from two speciose families (the Daphniidae and the Chydoridae) that vary widely in body size and phylogenetic distances (see electronic supplementary material). Cladocerans are in general quite similar in their main food sources, but there are clear differences in feeding habits of Daphniidae and the macrophyte-associated Chydoride (Barnett et al. 2007). The eight species that we used in our experiments are widespread and common in Belgian ponds and shallow lakes (Louette et al. 2007) (see also supplementary material). In order to test the influence of trait and phylogenetic diversity on top-down control in zooplankton communities independently of species richness, we held species richness constant at two per microcosm.

We measured grazing rates of different zooplankton pairs that represented four crossed levels of body size diversity (functional trait diversity; FD) and phylogenetic diversity (PD) (Fig. 2). The four treatments were: (i) HIGH FD – LOW PD; (ii) LOW FD – HIGH PD; (iii) HIGH FD – HIGH PD; and (iv) LOW FD – LOW PD. For each of these four treatments there were two pairs of species, and for each species combination there were three replicates (for details see electronic supplementary material, figure S2 and table S2). This orthogonal experimental design allowed us to test whether variation in grazing rates occurred mainly along the trait axis, mainly along the phylogenetic axis, or rather depended on the combined information of both axes (Fig. 2). It is important to note that different communities may have exactly the same trait diversity pattern (i.e., homogeneous size distribution; LOW FD) but differ considerably in average size (e.g., represented by communities A and C in figure 1c). For the two

treatments involving LOW FD [i.e., treatments (ii) and (iv), with low variation in body size] we contrasted two large versus two small species (see electronic supplementary material for details, table S2). This design allows us to separate the effects of community average size (i.e., large versus small; communities A and C in Fig 1c) from the effects of size diversity (represented by community B in Fig. 1c).

All species were collected from communities sampled in farmland ponds and shallow lakes in Belgium, and were isolated and cultured as single clones. The experimental populations of all species were cultured for multiple generations under standardized conditions (in 500-ml glass jars, dechlorinated tap water, 20 °C, 14 h light/ 10 h dark photoperiod) prior to the grazing experiments. All studied cladoceran species are cyclical parthenogens. They produce a number of clonal offspring in intervals of approximately two to four days. For the grazing experiments we used cohorts with similar sized mature individuals to rule out trait variability within species. Body size values for adult individuals of each species were measured after each grazing experiment (see electronic supplementary material, Table S3). Average body sizes of the different species used ranged from 0.52 to 3.60 mm. The species were fed with a mixture (1:1 ratio) of two different algae species that vary considerably in cell size, *Chlorella vulgaris* (approximately 3 μm) and *Scenedesmus obliquus* (approximately 15 μm). Both algae species were cultured in isolation with the addition of trace elements.

Experimental setup

To measure feeding rates by zooplankton assemblages on algae, which

we use as a proxy for the capacity of top-down control on algae, we calculated the gross change in phytoplankton biomass in experimental units over a 24-hour period. Grazing experiments were carried out in clear 250ml glass Duran bottles. The number of individuals for each species per experimental bottle was standardized based on biomass (dry weight over 24h/70ºC) (see electronic supplementary material, table S3). To begin each grazing experiment, we transferred the required number of individuals (see electronic supplementary material, table S3) to experimental bottles then added an algal solution with two species, the small algae *Chlorella vulgaris* and the large *Scenedesmus obliquus* in equal proportions (1:1) with an initial concentration of approximately 150.000 cells per ml. We choose those algae species because they are widespread and common in freshwater systems, easy to culture under standardized laboratory conditions and because they vary considerably in cell size. Bottles were placed in a roller machine (four turns per min. along the longitudinal axis of the experimental jars) to avoid algae settlement during the grazing experiment. Control bottles (n = 3 per experiment) were identical to experimental bottles but had no zooplankton. We also performed grazing experiments for all species in monocultures replicated three times.

At the start and at the end of each grazing experiment, we took a 2 ml water sample from the centre of experimental and control bottles and fixated with 110 µl formalin to count algae cell numbers afterwards. Cells were counted with the Attune® Acoustic Focusing Cytometer (Applied Biosystems™ by Life Technologies™, Carlsbad, CA, USA), which allowed us to measure the total number of algae

cells per ml and to differentiate between small and large algae cells. The latter information was crucial to test whether large zooplankton species are more efficient grazers on large phytoplankton while smaller zooplankton species are more efficient grazers on small algae cells, thus leading to size-related complementarity via diet niche partitioning (Cyr and Curtis 1999, Ye et al. 2013).

After each grazing experiment, we removed the zooplankton from the bottles and measured total body length (excluding tail spines) of 10 individuals from each replicate using a dissecting microscope. For larger species with less than 10 individuals per replicate (see electronic supplementary material, table S3), all the individuals in a replicate were measured. To compare grazing rates (GR) among zooplankton assemblages, we calculated phytoplankton consumption over the period of 24-hours.

Statistical analysis

We calculated grazing rates as the total gross change in phytoplankton biomass using the following equation:

$$GR = [(\Delta \text{ Phytoplankton control}) - (\Delta \text{ Phytoplankton treatment})] \times \text{Volume}$$

where "Δ Phytoplankton control" is the logarithmic difference between the phytoplankton concentration over 24-hours in the control jars (i.e, Control phytoplankton$_{t0}$ – Control phytoplankton$_{t1}$) and "Δ Phytoplankton treatment" is the logarithmic difference between the

phytoplankton concentration over 24-hours in the jars with zooplankton (i.e, Treatment phytoplankton$_{t0}$ – Treatment phytoplankton$_{t1}$). Using the same equation, we also calculated: (i) GR_{small} as the gross change in small phytoplankton (C. *vulgaris*) over 24-hours and (ii) GR_{large} as the gross change in large phytoplankton (*S. obliquus*) cells over 24-hours.

We first calculated phylogenetic and functional trait (body size) distances among all pairs of species used in the experiment. Phylogenetic distances among species were estimated from a reconstructed molecular-phylogenetic tree (based on Maximum Likelihood) for cladocerans occurring in Belgium (for details on the phylogenetic tree reconstruction see electronic supplementary material). Based on this phylogenetic tree, we calculated a phylogenetic (cophenetic) distance (PDist) matrix between all species pairs based on branch lengths (Swenson 2014). Functional trait distances among species, the Euclidean distance between the average measured body size values, were used to construct a functional distance (FDist) matrix.

For each distance matrix, we calculated the mean pairwise distance among species in a given assemblage using the package **Picante** in R (Kembel et al. 2010) (note that the mean pairwise distance for communities with only two species is equal to FDist and Pdist). This results in a measure of trait (FD) or phylogenetic (PD) diversity within experimental bottles. The diversity measures FD and PD may reflect trait or phylogenetic complementarity if species that vary in size or evolutionary relationships exploit alternative resources (Fig. 1b-c) (Cadotte 2013, Ye et al. 2013, Deraison et al. 2015). In

addition to the variance component of size (i.e., size diversity, FD) we also calculated community average size (hereafter CAS; Fig. 1c). CAS may reflect grazing efficiency asymmetry (i.e. a selection effect) if differences in grazing efficiency between large versus small species (Fig. 1a) among communities results in different top-down control (represented by solid line in Fig 1c).

As explained above, the information captured by FD, PD and CAS reflects different mechanisms of ecosystem function, i.e. complementarity (FD and PD) versus grazing efficiency asymmetry (CAS) (Fig. 1). Yet, they can also overlap in their role in explaining ecosystem functioning if they are correlated. For example, starting from a community composed of only small species (community C in Fig. 1c), both trait diversity and community average size can increase in the same direction (captured in the shift to community B in Fig. 1c). In order to quantify the unique and shared effects of community average size, community size diversity and community phylogenetic diversity on grazing rates, we applied variation partitioning based on partial regressions (Whittaker 1984, Borcard et al. 1992, Legendre and Legendre 2012) using PD, FD, and CAS as predictors, and grazing rate as a response variable. This statistical method separates the variation in a response variable(s) into independent contributions of predictors, shared contributions among all combinations of predictors (i.e., variation that is associated with multiple predictors, which results when predictors covary with one another), and unexplained causes of variation (Borcard et al. 1992, Borcard et al. 2011). The proportion of explained variation for each component is given by the adjusted R^2 ($_{adj}R^2$, an R^2 statistic adjusted to avoid type I error and consequent

overestimation of the amount of explained variation (Peres-Neto et al. 2006)). Because $_{adj}R^2$ is an estimate based on the number of variables included, negative values may occur but are interpretable as not significantly different from zero. The proportion of variation explained by each component is additive and sums to one. We considered each replicate separately and used trait-phylogenetic distances to control for non-independence among replicates (i.e., each replicate had the same value for CAS; FD and PD).

Zooplankton species show feeding niche complementarity if small zooplankton species are more efficient grazers on small algae cells and large zooplankton species are more efficient grazers on large algae cells (Fig. 1b (Cyr and Curtis 1999, Ye et al. 2013)). To test whether this was observed in our experiment, we classified each zooplankton species as large (>1.5 mm; n = 4) or small (≤1.5 mm; n = 4) then tested the effect of body size class on grazing rates (as observed in monocultures). To do this we ran three separate analysis of variance (ANOVA), using body size as a factor and three response variables, one at a time: (1) total grazing rates (i.e., the entire spectrum of phytoplankton cell sizes), (2) grazing rates on small algae cells, and (3) grazing rates on large algae cells.

Species interactions, such as facilitation and competition, may have non-additive consequences on ecosystem functions. To test the hypothesis that the performances of two-species communities equalled the average performance of the contributing species in monoculture we first calculated the expected grazing rates (GR_{EXP}) for each species pair by averaging the grazing rate values of each species in monoculture. We subtracted this from the observed grazing rates in

combination (Sp_{int} = GR_{OBS} - GR_{EXP}, where Sp_{int} refers to species interactions and GR_{OBS} refers to observed grazing rates for a given species pair. Thus, a value of zero indicates that observed grazing rates are equal to what would be expected for a given species combination without any interaction, whereas positive values indicate synergistic interactions, where the two-species communities perform better than the average of the two monocultures, e.g. because of niche complementarity. Negative values indicate antagonistic species interactions, e.g. due to interference competition. Values were transformed to percentages to allow comparisons among species pairs. We used two-way permutation univariate ANOVA (Anderson 2001) (package **lmPerm** in R) to test the effect of functional trait diversity (FD) and phylogenetic diversity (PD) on species interactions $Sp_{int.}$ All analyses were run in R v.3.2 (2015).

RESULTS

Variation partitioning revealed that the explanatory variables PD, FD and CAS and their intersections accounted for approximately 64% of observed variation in grazing rates (Table 1). Community average size (CAS) accounted for approximately 93 % of the total amount of explained variation, either as a pure effect (41% of explained variation) or shared with FD (52% of explained variation). Specifically, communities composed of larger species depleted algae more efficiently than communities composed of smaller species (see also electronic supplementary material, figure S4). The pure effects of PD and FD were not significant, whereas the pure effect of CAS was highly significant (Table 1). Additionally, FD and CAS shared a large

fraction of overlapping information (Table 1). Phylogenetic diversity was uninformative either as a pure effect or shared with other variables (Table 1).

Table 1. *Proportion of explained variation obtained through variation partitioning analysis based on partial regressions on top-down control given by each predictor and their shared effects. Predictors are as follows: PD = phylogenetic diversity; FD = functional trait (body size) diversity; and CAS = community average body size. Three asterisks represent p < 0.001. Note that it is not possible to calculate p-values for intersections, i.e., shared effects.*

Components	$_{\text{Adj}}\mathbf{R^2}$
pure effect of phylogenetic diversity (PD)	0.000
pure effect of functional (body size) diversity (FD)	0.043
pure effect of community average body size (CAS)	0.262***
shared effects between PD and FD	0.003
shared effects between FD and CAS	0.330
shared effects between PD and CAS	0.000
shared effects between PD, FD and CAS	0.000
Total explained variance	0.638 ***

The analysis of variance based on monocultures revealed that larger zooplankton species depleted both large and small phytoplankton more efficiently than small zooplankton (Fig. 3a-b) (large algae: $F = 103$, $p < 0.001$; small algae: $F = 19.42$, $p < 0.001$). The larger species were therefore superior grazers over the entire

spectrum of algae sizes ($F = 49.73$, $p < 0.001$) (Fig. 3c; see electronic supplementary material).

Table 2. *Two-way permutation ANOVA results, testing for the effect of phylogenetic diversity (PD) and functional diversity (FD) on species interactions (quantified as the deviation from the average performance of the species in monoculture) for grazing rates. Df refers to degrees of freedom; SS means sum of squares.*

Factors	*Df*	*SS*	*p*-values
PD	1	140.3	0.673
FD	1	4810.5	< 0.001 ***
PD : FD	1	2633.7	0.028 *

Tests of species interactions on observed grazing rates indicate that species combinations with high functional trait diversity performed better than expected from the measurements on individual species (Table 2, see also electronic supplementary material, figure S5). In contrast, low functional trait diversity resulted on average in more negative species interactions (Table 2, see also electronic supplementary material, figure S5). There was no clear effect of phylogenetic diversity on species interactions (Table 2, figure S5), but we found a significant interaction between functional and phylogenetic diversity on species interactions.

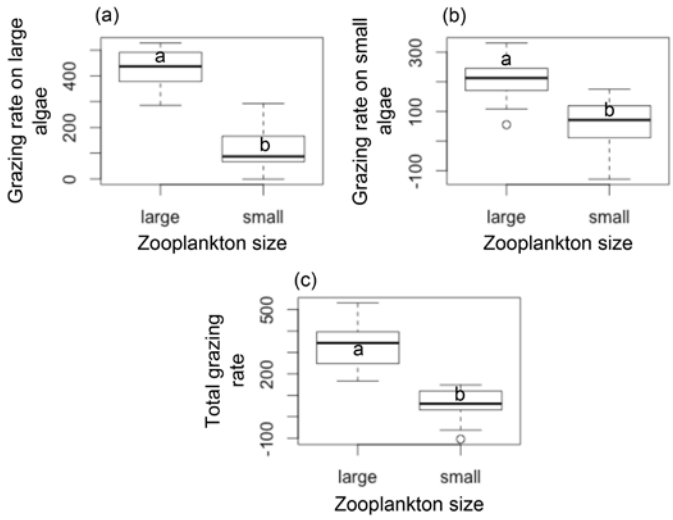

Figure 3. *Box plots depicting algae consumption by small and large zooplankton species on: (a) large algae cells; (b) small algae cells; (c) the entire spectrum of algae cell sizes. Distinct letters (a vs. b) indicate significant differences ($p < 0.001$) based on one-way ANOVA.*

DISCUSSION

Biodiversity metrics based on phylogenetic distances are increasingly being used as a surrogate for trait differentiation in ecosystem functioning research. While there is some support for this approach (Cadotte et al. 2008, Flynn et al. 2011), most of these examples are based on reanalysis of studies that were originally designed to test the effect of species richness on plant biomass production. As a consequence, previous studies had limited power to unequivocally assign the role of phylogeny independently of species and trait diversity, since all three biodiversity dimensions were somewhat

correlated (Flynn et al. 2011, Cadotte 2015, Venail et al. 2015) [but see Cadotte (2013)]. By varying trait and phylogenetic distances independently and holding species richness constant in our experimental study, we could disentangle the role of phylogeny and a key trait (body size) in determining zooplankton top-down control on phytoplankton, thus achieving strong inference power to detect the role of phylogeny if a key trait is accounted for. We clearly demonstrated that body size diversity, but especially community average size, were much better predictors of cladoceran grazing rates than phylogenetic diversity. We thus found no support for our first and second hypothesis that phylogenetic diversity could provide more accurate or complementary predictions of top-down control, respectively, than accounting solely for body size information in freshwater herbivorous zooplankton. We also demonstrated that large zooplankton species were superior grazers of both small and large phytoplankton cell sizes, indicating that the difference in grazing efficiency (selection effect) between small and large zooplankton species (Fig. 1a) was more important than niche differences (complementarity) (Fig. 1b) in mediating trophic interactions in our experimental setting.

Studies detecting a relationship between species richness and ecosystem functions often observed a high variance in ecosystem functioning for the same number of species and could not always mechanistically explain this relationship (Cardinale et al. 2006, Cardinale et al. 2012). These variable results could derive from trait and evolutionary differences among species, which are ignored in taxonomic indices of diversity (Srivastava et al. 2012b, Deraison et al.

2015). Our study confirmed that top-down control of algae can differ substantially among species pairs and this can be explained by inter-specific trait differences. By systematically contrasting functional trait and phylogenetic distances, we demonstrate that variation in a single trait, body size, predicted grazing rates better than measures of phylogenetic distance in this system. Thus we found strong support for our third hypothesis that body size is a key trait determining cladoceran top-down control on unicellular algae and that phylogenetic distances do not add important information to predict grazing pressure in this system. A study of herbivorous marine amphipods where traits were not conserved along the phylogeny also found that directly accounting for traits provided better estimates of ecosystem function (resistance to invasion) than phylogeny (Best et al. 2013). However, our findings contrast with the results of (Thompson et al. 2015), who concluded that zooplankton phylogenetic diversity was a better predictor of top-down control than diversity in body size and other functional traits in freshwater shallow lakes. One potential explanation for this discrepancy could be due to the fact that the study of Thompson et al. [26] included copepods, whereas our study focused on variation within the cladocerans. Copepods are known to differ substantially in food niche from cladocerans (DeMott 1986, Barnett et al. 2007). In addition, it is often difficult to differentiate causes from consequences using observational data. Thus, two interpretations of the results of Thompson et al. (2015) are actually possible: phylogenetically diverse communities indeed reduced chlorophyll a more strongly in natural systems, or phylogenetically diverse

communities are more likely to occur in ponds with lower chlorophyll a.

Although some studies have demonstrated that phylogenetic distances can predict ecosystem functioning in experimental settings (Gravel et al. 2012, Cadotte 2013), it is important to note that phylogenetic distinctiveness is not an ecological mechanism *per se*. The use of phylogenetic distances as predictor of ecosystem functioning relies on the assumption that relevant traits are conserved along the phylogeny (Wiens et al. 2010). Ultimately, trait differentiation is the main currency linking biodiversity to ecosystem functioning (Hillebrand and Matthiessen 2009). Studies demonstrating that phylogeny is more important than the measured traits [e.g., (Cadotte et al. 2008, Cadotte et al. 2009)] either reflect a failure to correctly identifying which traits translate into enhanced ecosystem functioning or reflect that relationships are complex and thus cannot be captured by measuring just a few traits. Flynn and collaborators (2011) found that trait and phylogenetic distances complementarily explain ecosystem functioning, suggesting that measured labile traits and unmeasured phylogenetically conserved traits were both important in determining biomass production in grassland plants. In our system of herbivorous zooplankton, phylogenetic diversity became an important predictor of ecosystem functioning, along with diversity in body size, only after removing all cases of body size convergences and divergences and focusing exclusively on species combinations presenting trait conservatism (i.e., treatments (iii) and (iv) only; see electronic supplementary material). This illustrates that the potential for phylogenetic distances to predict top-down control

depends entirely on body size conservatism and does not add information on relevant unmeasured traits. On the other hand, our results also show that under such circumstances phylogenetic diversity is indeed superior to taxonomic diversity in explaining ecosystem functions.

Niche differentiation (complementarity) in resource use is often invoked to explain the relationship between biodiversity and ecosystem functioning. Another plausible mechanism, however, is selection effects, where more diverse communities are more likely to include at least one dominant species (Hector et al. 2002, Carroll et al. 2011). A recent observational study found that zooplankton body size diversity enhanced top-down control and suggested that this occurred because of niche partitioning in resource use (Ye et al. 2013). Ye et al. (2013) suggest that such a relationship emerged because smaller zooplankton potentially consumed smaller algae more efficiently while large zooplankton predominantly grazed upon large phytoplankton. Our experiments demonstrate that the same relationship between size diversity and grazing rates can emerge via grazing efficiency asymmetry (Fig. 1a) in the absence of niche differences. This occurs because in order to have a higher value for the index of size diversity, at least one large zooplankton species must be present, and these tend to be superior grazers. In the present study we used variation partitioning and demonstrated that accounting for community average body size rather than size diversity within assemblages (FD), enabled us to better predict differences in top-down control. Specifically, communities composed by larger species performed better than communities of smaller species. The majority of

studies relating trait and phylogenetic diversity to ecosystem functioning have only used variance metrics, such as FD and PD, as predictors and often suggested niche partitioning as the main mechanism determining ecosystem functioning (e.g., (Cadotte 2013, Venail and Vives 2013, Thompson et al. 2015)). As we have shown, combining variance and mean trait metrics enables better insight on the relative importance of complementarity and selection effect as determinants of ecosystem functioning (Deraison et al. 2015).

Our results also demonstrate that species interactions often have non-additive consequences on ecosystem functioning. Overall, we observed that functionally dissimilar species presented positive interactions (facilitation), while species overlapping in body size tend to present antagonistic interactions, probably due to interference competition. In *Daphnia*, reduced grazing rates among species has been shown to result from chemical and mechanical inhibitory mechanisms (Hargrave et al. 2011). Although we do not have data to further test this, our results support the idea that whatever inhibitory mechanisms of competition are at play, they are stronger among species that overlap in body size. Our study thus suggests that size-related grazing efficiency and size-related complementarity co-determine zooplankton top-down control on algae, but with a more important role for the former mechanism.

We used microcosm experiments to investigate the effect of functional and phylogenetic distances on top-down control. Several recent studies provided evidence that results from micro-scale BEF experiments are often representative of large-scale natural systems (Maestre et al. 2012, Duffy et al. 2015). The mechanisms on which

our interpretation relies, which are based on trait-based selection effects and non-additive species interactions, can be easily extrapolated to other systems. Our findings may apply directly to herbivorous marine zooplankton (Best et al. 2013) and to herbivorous terrestrial insects (Deraison et al. 2015), in which the impact of key traits on the biomass of lower trophic levels has been recently demonstrated. Additionally, our results may also be representative of other ecosystem functions, such as pollination, in which competitive asymmetry plays a larger role than complementarity (Winfree et al. 2015). The experimental approach we used, separating the role of a key functional trait and phylogenetic distances, provides exciting and promising avenues to understand how evolutionary convergences and divergences affect trophic interactions and shape ecosystem functioning. A potential limitation of our study was that it was not possible to design it so as to completely separate the role of community average size from that of phylogenetic composition. However, we performed *ad hoc* analysis and found that phylogenetic composition *per se* is not the driving force of top-down control in our experimental setting (for a full analysis of the effect of phylogenetic composition, see electronic supplementary material, figure S6).

In order to effectively inform management in an era of accelerated extinction rates, it has been suggested that BEF research should shift from approaches based on the number of species to more accurate trait and/or evolutionary-based approaches (Hillebrand and Matthiessen 2009, Srivastava et al. 2012b, Deraison et al. 2015), which could provide more mechanistic understanding of ecosystem functioning. Our study partially supports this idea, as we have shown

that body size diversity, and especially community average size, provided strong predictions of top-down control, despite constant species richness. This happens because large species are superior grazers across a range of algae cell sizes (Fig. 1a). Yet, we refuted our first two hypotheses, which are based on the idea that additional, evolutionarily conserved traits could provide added power to explain top-down control on phytoplankton. Our results illustrate the importance of pinpointing specific relevant traits to mechanistically understand and predict ecosystem functioning. Zooplankton top-down control on phytoplankton represents an important ecosystem service worldwide by controlling algal blooms, including toxic algae. Recent studies have demonstrated that increasing anthropogenic impacts, such as climate warming and chemical pollution will affect the size structure of zooplankton communities, potentially leading to a predominance of smaller zooplankton (Moss et al. 2011). Based on our results we suggest that to maintain zooplankton top-down control on algae, ecological conditions that favour combinations of larger, superior grazer species should be prioritized. One mechanism to do so is to reduce fish predation pressure (cf. biomanipulation as a management strategy (Moss et al. 2011)). In summary, our study provides evidence for strong trait-based top-down control and warns against a blind use of phylogenetic and taxonomic diversity as surrogates for trait differentiation to predict and explain ecosystem functions.

AUTHORS' CONTRIBUTION. AG and LDM developed the ideas and the concept of this study. AG designed the experiment with input

of LDM. AG conducted the experiment and performed the statistical analyses. JP and LDM contributed to discussions on data-analysis and interpretation. AG wrote the first version of the manuscript and all authors contributed substantially to the final version.

Funding

AG received a doctoral scholarship from Brazil through "Science Without Borders - CNPq", grant number 245629/2012-2, and JHP a postdoctoral fellowship (F+) from the KU Leuven Research Fund. Financial support was obtained from KU Leuven Research Fund Excellence Center financing (PF/2010/07), ERA-NET BIOdivERsA project TIPPINGPOND (nationally funded by Belspo) and Belspo IAP SPEEDY.

Ackowledgments:

We thank Berthe Vastenhoud and Luz Amadei for their help with data collection during the experiment. Patrick Venail, Pieter Lemmens and an anonymous referee provided valuable comments on an earlier version of the manuscript.

Supplementary information

Appendix S1. Detailed description of methods and supporting statistics.

GENERAL DISCUSSION

The current geological epoch has been termed the Anthropocene, which is characterized by increased global impacts on earth's ecosystems caused by human activities (Steffen et al. 2011). Amongst others, humans have altered natural ecosystems via increased pollution, habitat destruction, overexploitation and the loss of biodiversity (Zalasiewicz et al. 2011, Pimm et al. 2014, Allan et al. 2015). Additional concern arises from nutrient input in aquatic ecosystems and land use changes resulting from agriculture and urbanization (Kaye et al. 2006, Ulén et al. 2007), which have been shown to alter biodiversity patterns across spatial scales (Urban et al. 2006, Knapp et al. 2008, Knapp et al. 2012).

There is now a growing consensus that biodiversity positively affects a variety of ecosystem processes, such as decomposition rates, primary productivity and the control of algal blooms in shallow lakes (Cardinale et al. 2006, Cardinale et al. 2012, Lefcheck et al. 2015, van der Plas et al. 2016). In addition, biodiversity has also been linked to human well-being and to the provision of ecosystem services to our society (Naeem et al. 2009). Adequate management of ecosystems is needed if we are to maintain current levels of biodiversity and ecosystem services in the future.

Biodiversity is a multifaceted concept that requires a multi-scale approach to be fully understood (Segre et al. 2014). In addition to biodiversity quantification across spatial scales, biodiversity can also be quantified at different dimensions, such as taxonomic, functional trait or phylogenetic diversity (Weinstein et al. 2014, Liu et al. 2016).

In order to properly manage different biodiversity dimensions across spatial scales, it is therefore crucial to understand what are the drivers of biodiversity and at what spatial scale they act on different dimensions of biodiversity. In this thesis, I conduct such integrative research by investigating the drivers and consequences of different dimensions of biodiversity (i.e., taxonomic, functional and phylogenetic) across spatial scales.

The main goal of this PhD research was to quantify the relative importance of local and regional processes on different biodiversity dimensions (chapters I to III) and to investigate how such biodiversity modifications are likely to scale up and affect a key ecosystem process in aquatic systems, namely zooplankton top-down control on algae (chapter IV). We also aimed at understanding how increasing urbanization affects multiple dimensions of biodiversity across several spatial scales (chapter II) and how trait-spatial scale mismatches affect our capacity to analyze metacommunity assembly (chapter III). We used a combination of experimental and fieldwork approaches, which provided us complementary insights into the drivers and consequences of biodiversity under a scenario of increasing anthropogenic pressures on earth.

1. Biodiversity dimensions across spatial scales

In Chapter I we demonstrated the importance of separating beta-diversity into its nestedness and replacement components in order to link community assembly processes to biodiversity patterns across spatial scales. Particularly, we have shown that the interaction between dispersal and landscape-wide environmental heterogeneity influenced each component of beta-diversity in divergent ways. Such important interaction would never have been appreciated if the analysis had focused only on total beta-diversity, which is a common practice in metacommunity studies. In Chapters II to IV we extended the findings of Chapter I by also looking at different dimensions of biodiversity, namely functional trait and phylogenetic dimensions.

Functional traits can be used either as response or explanatory variables in ecological analysis (Díaz et al. 2013, Gerhold et al. 2015, Wu et al. 2016). On the one hand, when used as a response variable, researchers often aim to quantify the relative importance of assembly processes on biodiversity patterns (Chapters II and III) (Webb et al. 2002, Graham and Fine 2008, Graham et al. 2009). Such trait-based metacommunity approach has been hypothesized to be more informative and general than the more traditional, taxonomy-based approach that treats all species as equally differentiated (McGill et al. 2006, Messier et al. 2010, Spasojevic et al. 2014, Liu et al. 2016). We tested this idea along gradients of urbanization and land-use intensity (Chapters II and III). Indeed, we found that the explained variance provided by the measured environmental and spatial factors was substantially higher for trait-based approaches than for more

traditional, taxonomic based approaches (Chapters II and III). This implies that species responses to environmental and spatial factors are largely trait-mediated instead of species specific, indicating that different species with similar traits tend to respond in a similar way to environmental conditions. On the other hand, when using traits as explanatory variables, researchers often seek to attain more accurate predictions of diversity-ecosystem functioning relationship (Cadotte et al. 2008, Flynn et al. 2011, Srivastava et al. 2012a, Cadotte 2013). We also found that trait-based approaches indeed provide much stronger and accurate predictions of ecosystem function than species diversity (Chapter IV).

The power of trait-based ecological approaches greatly depends on which traits are measured and included in the analyses (Baraloto et al. 2012, Cadotte et al. 2013, Díaz et al. 2013). Whereas this choice is made *a priori*, ecologists often have a good comprehension of their study system and about good candidate traits to be measured. For instance, zooplankton body size has been repeatedly suggested to be a major trait mediating both species responses to environmental and spatial factors as well as species effects on ecosystem processes (Brooks and Dodson 1965, Dodson 1974, Ye et al. 2013, Thompson et al. 2015). Overall, our results support this idea as we have shown that zooplankton species responses along urbanization gradients are size-mediated, with larger species being more negatively influenced by urbanization (Chapter II). Moreover, we have shown that body size variation within communities varies considerably along a gradient of pH, presumably due to differences in productivity, which may influence size-related species interactions (Chapter III) (Dodson et al.

2000). Furthermore, we demonstrated a very strong link between zooplankton body size and grazing efficiency in our experimental settings of microcosms (Chapter IV). Specifically, larger species tend to be superior grazers on algae, implying that the negative effect of urbanization on larger zooplankton species (Chapter II) will likely affect ecosystem processes, such as top-down control of algae and herbivorous biomass production (Thompson et al. 2015). It is important to bear in mind, however, that the reliance of body size as a major response and effect trait does not mean that the incorporation of additional traits would not be necessary to quantify species responses along ecological gradients and their effects on ecosystem processes (Barnett et al. 2007, Vogt et al. 2013). For instance, in Chapter III we show that the importance of body size as a structuring factor strongly depends on the environmental context and on the spatial scale considered. Particularly, body size was found to be a very informative trait for α- but not for β-diversity analysis, whereas the trait plant association was very informative for β-diversity but lacked power to inform α-diversity analysis (Chapter III). In a similar way, we have shown in Chapter II that there was a strong association between urbanization and body size turnover. Yet, when focusing on patterns along several environmental and spatial gradients, other traits complemented the information provided by body size, so that the explained variance increased substantially when considering multiple traits in combination (Chapter II).

The fact that the informative content of the measured traits varies depending on the environmental and spatial scales of interest makes it difficult to choose *a priori* which traits should be included in a trait-

based analysis (Chapters II and III). Moreover, even if we have good candidate traits to be included in the analysis, it is often unfeasible to measure all relevant traits for multiple species at the metacommunity scale (Baraloto et al. 2012). Such potential limitations of trait-based studies lead some researches to suggest an alternative approach, using species evolutionary distances as a proxy of functional differentiation (Webb et al. 2002, Graham and Fine 2008). This idea is based on the observation that more related species tend to share more functional attributes than in comparison to distant relatives (Blomberg et al. 2003, Wiens et al. 2010). Accordingly, phylogenetic distances among species could provide a synthetic representation of species similarities and differences in multiple traits, such as physiological traits, diet, size, colour and many other traits. We tested this idea by comparing the performance of phylogenetic-based approaches with that of taxonomy- and trait-based approaches in metacommunity analyses (Chapters II and III) and ecosystem functioning research (chapter IV).

Overall, we found that phylogeny-based approaches are more informative than taxonomy-based approaches but results based on phylogeny-trait comparisons were very mixed across chapters. This indicates that the power of phylogeny in comparison to trait-based approaches is not conclusive and may depend on many factors, including the spatial scale of the observations (Chapter III) and on whether or not specifically relevant traits present a phylogenetic signal (Chapters II and IV) (Wiens et al. 2010). For instance, in Chapter II all measured traits presented a strong phylogenetic signal so that the power of multiple traits and phylogeny to inform a metacommunity analysis was very similar. By contrast, in Chapter IV we used an

experimental design in which body size differences and phylogenetic distances varied independently from each other to test whether and how each diversity dimension influences a key ecosystem function, zooplankton top-down control of algae. Results from this experiment demonstrated that body size is a major trait determining grazing efficiency whereas phylogenetic distances were uninformative. It is noteworthy that, when we removed from the analysis treatments in which body size appeared to be converged or diverged and focused only on species pairs that present a pattern of phylogenetic signal in body size, then phylogenetic distances became as important as body size to predict top-down control in our experimental settings (See appendix S1 of Chapter IV).

The fact that phylogeny was informative in our experimental study **only when body size presented a phylogentic signal**, indicates that phylogenetic distances did not capture additional ecological information relevant to predict this ecosystem function. This highlights the fact that phylogenetic diversity is not an ecological mechanism *per se*, but rather that the information provided by phylogeny largely depends on which traits present a phylogenetic signal (Best et al. 2013). Therefore, depending on phylogenetic signal (or lack of it), multiple scenarios are possible for the relationship between phylogeny and function, which results in high uncertainty in predictions of ecosystem functioning based on phylogenetic distances alone. First, if all measured traits present a phylogenetic signal, then trait and phylogenetic distances will be highly correlated and provide similar explanatory power. Secondly, to the extent that measured traits are labile, directly measuring traits may provide superior power to

predict ecosystem functions than phylogeny (Chapter IV; see also Best et al. 2013). Thirdly, if phylogenetic distances capture information on unmeasured relevant traits, it is likely that phylogeny will complement the information provided by the measured (labile) traits. This was exemplified by a study in grassland ecosystems, where several measured traits were labile but informative and phylogenetic distances provided additional information to predict primary productivity (Flynn et al. 2011). Finally, if phylogeny captures information on the right traits whereas the measured traits are not informative, then the power of phylogeny to predict ecosystem function will be superior to that of species diversity and measured traits (Cadotte et al. 2008). The latter situation, has been demonstrated in a few studies, which set the stage for a number of phylogenetic-based studies aimed at predicting ecosystem functions both in terrestrial and aquatic ecosystems (Cadotte 2013, Venail and Vives 2013, Thompson et al. 2015). Our study provides a more cautionary perspective on the blind use of phylogenetic distances to predict ecosystem functioning and suggests that comparing both trait and phylogenetic approaches are needed to scale the explanatory power of the measured traits against whatever unmeasured traits that phylogeny might represent.

The power of our experimental approach (Chapter IV), manipulating trait and phylogenetic distances independently, is that we could disentangle the effect of a key functional trait and phylogeny while keeping species richness constant. This has been a recurrent problem in the literature, because most recent studies aimed at testing the relative power of functional trait diversity and phylogeny on

ecosystem functions were based on re-analysis of studies originally designed to test the influence of species richness on ecosystem functions (Venail et al. 2015). Consequently, most previous studies had limited power to unequivocally assign the role of phylogeny independently of trait and species diversity, since all three measures were somewhat correlated (Cadotte 2015, Venail et al. 2015). We encourage researchers to run more experiments similar to ours with other organism groups in more natural systems in order to identify the links between key traits and ecosystem processes, using phylogeny as a benchmark to scale the explanatory power of measured traits and to develop hypotheses about additional traits that might be captured by phylogenetic distances. In this sense, it is also important to bear in mind some potential limitations of our study (Chapter IV), which we recommend should be avoided in future studies. First of all, we used only two species because of the restrictions associated with the number of trait and phylogenetic combinations that are feasible to manipulate in our study system. This limitation was mainly due to the reduced number of Belgian cladocera species that we could keep in healthy conditions in the laboratory to create the scenarios of trait convergence, divergence and phylogenetic signal. However, we are aware of at least one study that manipulated species richness and phylogenetic distances independently and reported an important interaction between these two variables on ecosystem processes (i.e., bacteria decomposition rates) (Venail and Vives 2013). A second limitation concerns the simplified environmental conditions and short time span of our microcosm experiment. More specifically, we used only two edible green algae species, and we kept temperature constant

over the period of 24 hours. In hindsight, this may have limited our ability to detect diet partitioning and species complementarity to environmental change, so that our conclusion that phylogeny was not informative may reflect the simplicity of our experiment. In addition, trait diversity was somewhat correlated with trait composition, which should be avoided in future studies. More studies are therefore necessary to test to what extent our results are representative of more natural systems characterized by diverse phytoplankton communities, including toxic algae, and subject to environmental fluctuations. Furthermore, studies that manipulate trait and phylogenetic distances independently over a much longer time span are needed to test how each diversity dimension influences ecosystem stability and the stability of functions, where we expect the role of complementarity to be greater than that of selection effects (Cardinale et al. 2007).

2. Insights from integrated trait-phylogenetic approaches

Overall, we observed that trait- and phylogenetic-approaches increase substantially the explanatory power provided by environmental and spatial processes on metacommunity assembly compared to more traditional, taxonomy-based approaches (chapters II and III). This supports the idea that trait and phylogenetic information can approximate species similarities and differences that are key in determining biodiversity patterns across spatial scales. However, there are potential limitations of approaches that account only for trait or only for phylogenetic information, which we explored in more detail in chapter III. On the one hand, trait-based approaches rely on the

assumption that the most relevant trait(s) for a given environmental and spatial context were properly measured. This is tricky because the same trait that is informative at a given spatial scale or along a given environmental gradient may be uninformative at other spatial and environmental contexts (chapter III). On the other hand, it is a key limitation of approaches that consider only phylogenetic information that one cannot distinguish among different traits that are associated with different environmental gradients or spatial scales. As a step forward, we developed a conceptual framework for integrating trait and phylogenetic information in order to maximize the explanatory power and interpretability of environmental and spatial processes on community assembly patterns (chapter III). Specifically, we propose the use of phylogenetic information as a benchmark to assess the explanatory power of different traits across spatial scales. Applying our framework to a metacommunity of 91 ponds and shallow lakes revealed that body size is an important α-niche trait, strongly responding to processes at the α-scale, but it is not very informative to processes at the β-scale. Conversely, the trait plant association was very informative to β-scale processes but was uninformative to α-scale processes. Importantly, combining both traits into a single metric resulted in suboptimal explanatory power for both, α- and β-scale processes. This implies that several previous studies that have blindly combined different traits to assess functional patterns across spatial scales or along different environmental axes may have introduced potential confounding effects into the analysis. Furthermore, we observed that phylogeny-based analyses behaved similarly to analyses that combine both α- and β-niche traits, suggesting that both α- and β-

niche axes were conserved along the phylogeny. A future re-analysis is needed to reveal the extent to which ignoring confounding effects among α- and β-niche traits may have resulted in misleading interpretation of community assembly processes in previous studies.

3. Drivers of biodiversity patterns across spatial scales: dispersal, nutrients and urbanization

Our re-analysis of an experimental metacommunity dataset indicates that nutrient input in freshwater ponds can strongly reduce taxonomic diversity locally (i.e., α-diversity; chapter I). This finding is in line with a number of previous studies that reported species losses after nutrient inputs in both aquatic and terrestrial ecosystems. In addition, we found that high nutrient input resulted in β-diversity via a nested pattern in unconnected landscapes, indicating that only a few species from the regional pool can cope with high nutrient concentrations (chapter I). Taken together, those findings illustrate how the effects of nutrient addition on biodiversity propagate across spatial scales, affecting both α- and β-diversity in synchrony. This is a non-trivial result since there are several cases in which environmental change results in high species replacement (i.e., β-diversity), but with no effects on α-diversity. Furthermore, our field survey also suggests that nutrient input in ponds and shallow lakes, particularly phosphorus, strongly influences functional trait and phylogenetic β-diversity of zooplankton (chapter II). More specifically, we observed that high nutrient concentrations were associated with dominance of large bodied cladocerans of the Daphniidae family. This implies that

phosphorus input not only affects species diversity within and among communities but also influences different dimensions of zooplankton metacommunity structure (i.e., functional trait and phylogenetic dimensions; chapter II). Another study has similarly suggested that high nutrient concentrations may result in the dominance of large zooplankton and a reduction in taxonomic species diversity (Dodson et al. 2000). A potential explanation for this is that large zooplankton species are superior grazers (chapter IV) and can competitively exclude smaller species from systems with high nutrient concentrations (Brooks and Dodson 1965). Another explanation is that nutrient addition results in a shift in phytoplankton community composition – from small to large algae species – which results in habitat filtering against small zooplankton species in those systems (Dodson et al. 2000). Large phytoplankton is inedible to small zooplankton species, so that small zooplankton may not be able to persist in those systems because of food limitation (i.e., low food quality despite of high phytoplankton biomass). Further studies are needed to clarify whether high nutrient concentrations results in competitive exclusion of small zooplankton species by large ones (competition hypothesis) or rather results in habitat filtering mediated by changes in phytoplankton size structure (habitat filtering mediated by bottom-up effects hypothesis). The two mechanisms can be disentangled experimentally. For instance, one can increase the nutrient content of algae whereas keeping phytoplankton community composition constant, and then perform competition experiments among zooplankton species that range considerably in size.

Whatever mechanisms of community assembly are at play (either competition or habitat filtering), it is clear that phosphorus addition reduces taxonomic diversity of different organism groups, both in aquatic and terrestrial systems (Hautier et al. 2009, Kruk et al. 2009). This can be problematic because biodiversity loss resulting from eutrophication has been linked to a reduction of ecosystem functioning and stability (Hautier et al. 2015). However, it is noteworthy that in cladocera zooplankton body size has been demonstrate to be a key functional trait, enhancing top-down control of algae (chapter IV) and secondary biomass production (Thompson et al. 2015). Therefore, it is possible that by increasing the dominance of large zooplankton species (Chapter II), nutrient addition may actually enhance zooplankton top-down control of algae despite high levels of zooplankton species extinctions locally (Chapter I). This is likely to happen because of a negative relationship between the response and the effect (Larsen et al. 2005) of body size to nutrient input. Specifically, smaller zooplankton species are the first to be eliminated following nutrient addition (strong response) (chapter II) but those species are also functionally less important (weak effect) (chapter IV). Conversely, large, superior grazer species actually benefit from phosphorus addition (chapter II). Consequently, this might lead to a negative relationship between taxonomic diversity and ecosystem functions following nutrient addition (i.e., few large species may sustain higher levels of function than many small ones), although more studies are needed to investigate this hypothesis. After a certain threshold is surpassed, however, the effects of nutrient input becomes prejudicial even to large zooplankton species, resulting in a sudden

collapse of ecosystem processes and a state shift from clear water to turbid conditions, characterized by blooms of toxic algae (Scheffer 2004).

Whereas nutrient addition had a negative effect on local diversity (Chapter I) and a positive effect on zooplankton size composition (Chapter II), we demonstrate experimentally that dispersal had a positive effect on zooplankton diversity (chapter I). This suggests that high dispersal has the potential to counteract the negative effect of nutrient addition on zooplankton diversity (see also Shurin 2001). In addition, high dispersal reduced the contribution of the nestedness component and enhanced the contribution of the replacement component (turnover) to β-diversity in heterogeneous landscapes (Chapter I). This finding is in agreement with a number of observational studies that have suggested a link between spatial isolation and metacommunity nestedness and between high connectivity and species replacement (Fernandes et al. 2013, Henriques-Silva et al. 2013, Bender et al. 2016). Our results imply that enough dispersal is needed to maintain high levels of species complementarity at the metacommunity scale and high levels of species diversity at the local scales [see also (Shurin 2001, Symons and Arnott 2013)]. Nonetheless, we observed distinct patterns of nestedness and replacement across dispersal levels in experimentally homogeneous landscapes (Chapter I). Specifically, reduced dispersal resulted in more species replacement in experimental homogeneous landscapes, whereas increasing dispersal reduced species replacement. This indicates that high dispersal increases species complementarity among local habitat patches in heterogeneous landscapes via species

sorting, whereas lack of connectivity increases species complementarity in homogeneous landscapes via stochastic drift. From a conservation perspective, high nestedness implies the need of preserving the most species richer sites in order to effectively preserve the regional species pool. In contrast, high species replacement would demand the preservation of multiple sites along environmental gradients to maintain regional species diversity (Angeler 2013). Our results suggest that spatial connectivity is essential to enhance local and regional diversity along environmental gradients (via species replacement; chapter I), as has been repeatedly suggested in many observational studies.

Whereas our results from Chapter I are in clear concordance with both previous observational studies and with our conceptual framework we also acknowledge some potential limitations concerning our experimental approach and our simplified conceptual scheme. First of all, experimental metacommunities represent only a simplified abstraction of “real” metacommunities. In our experiment, dispersal rates were a function of species relative abundances and not a reflection of species dispersal traits. Although this is a common situation for passive dispersing organisms, we acknowledge that different dispersal mechanisms could have lead to different conclusions (Grainger and Gilbert 2016). Experimental dendritic networks allow species to disperse actively and represent an interesting alternative to quantify the influence of dispersal on diversity patterns within and among communities (Altermatt et al. 2015). More experimental studies with dendritic networks are needed to test to what extent our interpretation of the drivers of replacement

and nestedness are robust to different dispersal mechanisms. On top of that, our predictions (**see figure 1 in Chapter I**) are based on each scenario of dispersal level separately and we do not consider a situation in which some communities are more connected than others within the same metacommunity. We left this scenario out because of its high complexity, which hampers generalizations and makes it difficult to draw clear predictions. In our scheme, we assume that metacommunities are highly fragmented, moderately fragmented or highly connected and that such connectivity scenarios affect nestedness and replacement as depicted (Fig. 1 of Chapter I). It is important to note that many natural metacomunities often encompass a gradient of connectivity. As a result, less connected sites might form nested subsets of highly connected sites, but we do not consider this scenario because our experiment was not designed to tackle this situation. More studies are necessary to address this type of complexity. Dispersal gradients can also emerge as a function of species dispersal abilities, for instance if there is a trade-off between colonization capacity and competitive ability (Leibold et al. 2004). In addition, we assume that environmental heterogeneity is not nested (i.e., with smaller habitat patches containing only a subset of microhabitats present in larger patches). Therefore, whereas we acknowledge that our scheme has some inherent simplifications, it is still expected to be general for a number of situations in which homogeneous or heterogeneous landscapes are highly connected, moderately connected or highly fragmented, as depicted in our conceptual figure 1 in Chapter I.

In chapter II we found a significant pure spatial signal on functional trait and phylogenetic β-diversity. A potential limitation of our observational study (Chapter II) is that we used spatial descriptors as a proxy of dispersal rates rather than linking diversity directly to actual dispersal rates (as we did experimentally in Chapter I). Unfortunately, such an approach can be misleading because the pure spatial signal on diversity patterns can result either from dispersal limitation per se or from unmeasured environmental variables that are spatially structured (Peres-Neto et al. 2006). Therefore, we cannot interpret the pure spatial signal on trait and phylogenetic diversity patterns as an indicative of dispersal limitation along the urbanization gradient (Chapter II). Nonetheless, another recent study on beetles that was conducted in the same region (Flanders, Belgium) found evidence for selection against poor dispersers in urbanized areas (Piano et al. 2016) and others have already demonstrated a relation between increasing urbanization and connectivity loss (Urban et al. 2006). Therefore, it is at least possible that urbanization itself creates barriers to dispersal and in this way resulted in the pure spatial signal in our study (Chapter II). However, we advocate that more studies are needed to quantify explicitly how urbanization may influence dispersal rates of zooplankton and other organism groups in the field. This is an extremely important question as the effect of urbanization is unlikely uni-dimensional but rather dependent on synergistic effects among multiple stressors, such as fragmentation, pollution and temperature increase.

Previous studies with freshwater organisms have already demonstrated strong effects of urbanization on metacommunity

structure, but were mainly taxonomic-based (Urban et al. 2006, Concepción et al. 2015). In this thesis, we used a hierarchical sampling design and investigated how urbanization may be influencing different dimensions of metacommunity structure (i.e., taxonomic, functional and phylogenetic), and at what spatial scales urbanization is acting (Chapter II). Overall, we found a much stronger association between urbanization and functional trait and phylogenetic composition than between urbanization and taxonomic composition. Specifically, urbanized areas were dominated by small zooplankton species from the Chydoriidae family, which are scrapers and plant associated (at least considering the species present in the studied regional pool). Conversely, relatively larger species from the Daphniidae family, which are pelagic and filter feeders (again in our study), prevailed in less urbanized systems. Urbanization, therefore, may have indirect effects on ecosystem functioning via the elimination of larger species, which are superior grazers (chapter IV) and have higher rates of herbivorous biomass production (Thompson et al. 2015). Several studies have already reported drastic changes in environmental conditions following urbanization. Such changes include increased chemical and light pollution and higher temperatures in urbanized areas, the so-called “urban heat island” (Oke 1973, Li et al. 2011). This might explain why larger zooplankton species seem to be more vulnerable to urbanization (Chapter II), since many previous studies have reported a negative association between temperature, pollutants and zooplankton body size (Moore and Folt 1993). It is noteworthy that the only spatial scale in which urbanization actually influenced all biodiversity dimensions

significantly was 50m radius (i.e., the smallest spatial scale quantified). This indicates that managing environmental conditions locally in urban areas can mitigate the negative effects of regional urbanization on larger zooplankton species. Urban green space such as parks are known to alleviate the urban heat island effect, as they provide a microclimate thanks to the evaporation and shading effect of trees (Hamada and Ohta 2010). In this way they create a so-called Park cool-island (Kleerekoper et al. 2012), with the size of the green areas determining the magnitude of the cooling effect (Chang et al. 2007, Li et al. 2011). Given that body size is a very responsive trait along urbanization gradients and that large-bodied zooplankton are superior grazers that may be more efficient in reducing algal biomass, a useful conservation strategy is to create a belt of green area around each urban pond, which can potentially alleviate the heat-island effect and mitigate the physiological stress imposed by high temperatures on large zooplankton species (Brans et al. 2016, Moore and Folt 1993).

It is important to mention that several previous studies have already demonstrated the major role of zooplankton body size in aquatic food-webs (Brooks and Dodson 1965). Zooplankton body size not only determines grazing efficiency on algae (Chapter IV) but also vulnerability to predation pressure and other stressors (Brooks and Dodson 1965, Moore and Folt 1993). Despite the key role of predation in determining zooplankton size structure, in this thesis we intentionally avoided the effects of fish predation pressure in all chapters. For instance, we did not manipulate fish predation pressure in our experiments (Chapters I and IV). All the same, we selected fishless ponds (Chapters II and III) to be able to identify the effects of

urbanization and land-use intensity without the confounding effects of fish predation pressure. Certainly, high predation pressure would have either magnified or strongly reduced the magnitude of dispersal limitation (Chapter I), urbanization (Chapter II) and land-use intensity (Chapter III) on zooplankton size structure. For instance, high fish predation pressure in urbanized ponds could have accentuated the shift in zooplankton body size from rural to urban ponds, but we avoided this effect by sampling fishless ponds. In contrast, high fish predation pressure in more rural ponds could have erased completely the observed effects or urbanization on zooplankton size turnover. Future studies manipulating fish predation pressure independently in rural versus urban ponds are needed to test for an interaction between predation and urbanization on zooplankton size structure and food-web dynamics [for an example of such type of "natural experiments" please see Symons and Shurin (2016)].

It is also important to clarify some inconsistent results between chapters. For instance, in Chapter II we observed that body size was strongly conserved along the phylogeny whereas in Chapter III we report that this trait is labile. Clearly, the detection and interpretation of phylogenetic signal in traits depend on which species are present in the regional species pool. In Chapter III, for instance, we recorded 35 species and found many cases of convergences and divergences in body size (see evolutionary trait-gram; figure 4 in Chapter III). By contrast, in Chapter II we recorded only 23 species and there was no large Chydoriidae species (such as *Eurycercus lamellatus* or even the relatively large *Alona affinis*) or really small Daphniidae species recorded, so that all Daphniidae species were consistently larger than

all Chydoriidae, which resulted in phylogenetic signal in this trait (see also evolutionary trait-gram; figure 2 in Chapter II).

Overall, in this thesis we have demonstrated the importance of considering different dimensions of biodiversity in metacommunity analysis and ecosystem functioning research. Our analyses demonstrate that taxonomic, functional trait and phylogenetic data can provide complementary insights into the drivers of metacommunity assembly and to predict ecosystem processes. Different traits might respond in distinct ways to environmental gradients across spatial scales. The power of phylogeny similarly depends on the spatial and environmental context and on which traits have a phylogenetic signal. Given such complexities, it is nearly impossible to know *a priori* which traits are more informative as well as which diversity dimensions are redundant (i.e., correlated) or complementary. In this thesis, I make a strong case in favour of approaches that either compare different diversity dimensions (Chapter II and IV) or that integrates different dimensions (Chapter III). This is needed to better understand metacommunity assembly and ecosystem functioning. From a more applied viewpoint, this thesis suggests that increased anthropogenic pressures on earth, such as those associated with urbanization and land use intensity, are likely to affect different dimensions of zooplankton diversity in combination. Whereas high nutrient inputs may reduce species diversity locally and select for larger zooplankton species, high dispersal rates may enhance diversity locally and regionally via rescue effects and species sorting, respectively (Shurin 2001). This indicates potential synergistic effects between fragmentation and nutrient inputs on biodiversity, which is

likely to happen due to intensification of agricultural land use. Larger zooplankton species tend to be superior grazers that are more efficient in converting food into biomass production (Brooks and Dodson 1965). The observation that increasing urbanization potentially selects against larger zooplankton species suggests indirect negative effects of urbanization on ecosystem processes, such as zooplankton top-down control of algae and herbivorous biomass production. Nonetheless, the association between urbanization and functional trait and phylogenetic composition was significant only when urbanization was quantified at the smallest spatial scale (i.e., 50 m radius), suggesting that managing environmental conditions locally may counteract the influences of urbanization on different dimensions of biodiversity.

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY INFORMATION TO CHAPTER I

Appendix S1. This appendix is permanently deposited online and can be accessed electronically using the following link: http://onlinelibrary.wiley.com/doi/10.1002/ecy.1666/abstract

SUPPLEMENTARY INFORMATION TO CHAPTER II

Appendix S1

1. Sample collection and analysis

Each pond was sampled once. The duration of the sampling campaign was 43 days (i.e., from May to July of 2013). To avoid interference from an effect of sampling time and any directional change in environmental condition associated with it, the order of sampling along the urbanization gradient was randomized over the different plot levels. Each day three ponds of an individual regional plot, and thus representing three different urbanization classes within a given plot, were sampled (Fig. S1). Across days we randomized the plots to be sampled so that there was no bias with respect to region or urbanization level. This randomized sampling design proved effective, as we performed MANOVA analysis using sampling time as explanatory variable and detected no temporal effect on species composition (p = 0.84) or on the selected urbanization and

environmental variables ($p > 0.5$ for all variables). At each sampling occasion, we measured pH and conductivity with standard electrodes. Water transparency was determined with a Snellers tube due to the characteristically shallow depths of the ponds. Depth-integrated water samples were collected in an open water zone of the pond using a tube sampler (length 1.5 m; diameter 75 mm) and kept in the cold (4 °C) and dark until further analysis. To ensure correct sampling of the water layer, contact with vegetation or the sediment was carefully avoided during sampling. We also estimated the percentage of cover of four different vegetation variables, defined as: (1) submerse, (2) floating, (3) emergent vegetation, and (4) total vegetation cover. Water depth was measured with a graduated stick in the central part of the pond, as well as at 10cm of the edge of the pond. The depth-integrated water samples were used for the assessment of the concentration of chlorophyll a, nutrients (total phosphorus and nitrates, and available phosphorus and nitrates), alkalinity and some major ions (calcium, chloride and sulphate ions, water hardness). Sulphates, chlorides, calcium, alkalinity and hardness were measured following standard methods according to the Hach Water Analysis Handbook (HACH, 1992). Nitrate concentration was determined after filtration on a GF/F filter with a Technicon autoanalyser III. Total phosphorus concentration was measured with the ascorbic acid method after perchlorate digestion (Murphy and Riley, 1962). In situ Chlorophyll a concentrations were spectrophotometrically determined with a portable fluorometer.

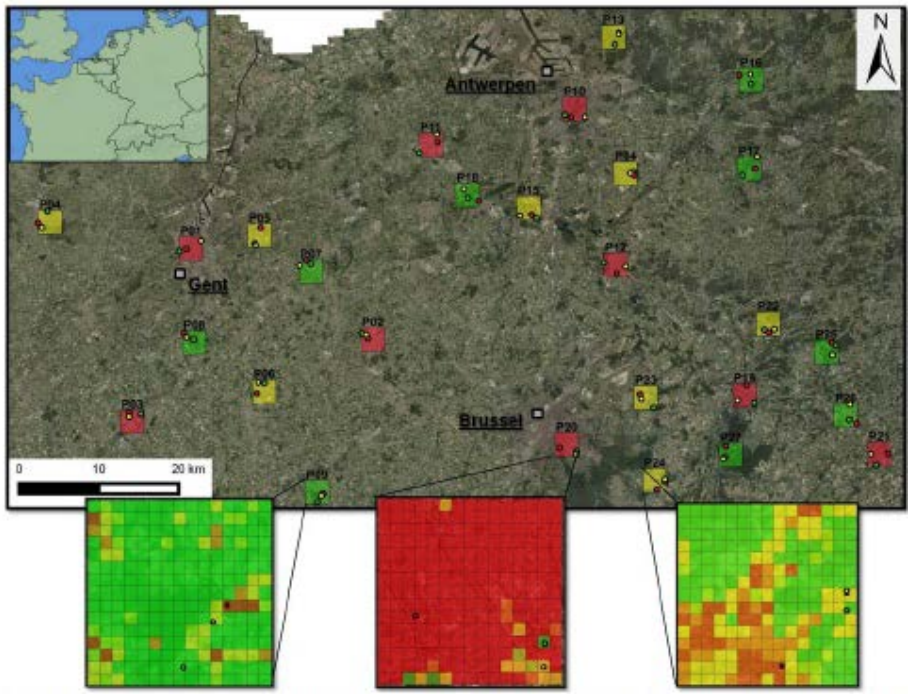

Figure 1: (a) *Map of Belgium (Flanders) with representation of the hierarchical design; large squares represent 3 by 3 km plots, with grids of 200m by 200m subplots within plots (bottom), locations of ponds indicated as small circles. Colors reflect urbanization levels: high (red), medium (yellow), and low (green) urbanization.*

2. Trait information

We extracted information on body size and plant association from the literature. Body size values are according to Alonso (1996). Values of the trait plant association were determined mainly based on Barnett et al. (2007) and complemented with information provided by Declerck et al. (2007) and Alonso (1996). Body size values were complemented with our own measurements, when available. The trait filtration type was mainly based on Barnett et al. (2007). However, we further

differentiated *Scapholeberis spp*. and *Simocephalus spp*. from the rest because those species do not belong to any of the categories presented in the literature. Trait values are given in Table S3.

Table S3: List of species recorded in our study and their respective trait values. Acronyms supplement figure 4 in the main text of Chapter II.

Species	Body size (mm)	Plant association	Filtration type	Acronym
Alonella excisa	0.4	1 (littoral)	1 (C-Filtration)	A.exc
Alonella exigua	0.35	1 (littoral)	1 (C-Filtration)	A.exi
Alona guttata	0.4	1 (littoral)	1 (C-Filtration)	A.gu
Alona rectangula	0.4	1 (littoral)	1 (C-Filtration)	A.re
Bosmina longirostris	0.6	3 (pelagic)	2 (B-Filtration)	B.lo
Ceriodaphnia quadrangula	0.9	2 (intermediate)	3 (D-Filtration)	C.qu
Ceriodaphnia laticaudata	0.9	2 (intermediate)	3 (D-Filtration)	C.la
Ceriodaphnia pulchella	0.8	2 (intermediate)	3 (D-Filtration)	C.pu
Ceriodaphnia reticulata	1.3	2 (intermediate)	3 (D-Filtration)	C.re
Chydorus sphaericus	0.5	1 (littoral)	1 (C-Filtration)	C.sp
Daphnia curvirostris	2.5	3 (pelagic)	3 (D-Filtration)	D.cu
Daphnia magna	4.0	3 (pelagic)	3 (D-Filtration)	D.ma
Daphnia obtusa	2.5	3 (pelagic)	3 (D-Filtration)	D.ob
Daphnia pulex	2.5	3 (pelagic)	3 (D-Filtration)	D.pu

Graptoleberis testudinaria	0.6	1 (littoral)	1 (C-Filtration)	G.te
Leydigia acanthocercoides	0.8	1 (littoral)	1 (C-Filtration)	L.ac
Pleuroxus aduncus	0.65	1 (littoral)	1 (C-Filtration)	P.ad
Pleuroxus denticulatus	0.6	1 (littoral)	1 (C-Filtration)	P.de
Pleuroxus truncatus	0.65	1 (littoral)	1 (C-Filtration)	P.tr
Scapholeberis mucronata	1.2	3 (pelagic)	4 (Scaphol. type)	S.mu
Scapholeberis kingi	1.6	3 (pelagic)	4 (Scaphol. type)	S.ki
Simocephalus exspinosus	2.8	1 (littoral)	5 (Simoc. type)	S.ex
Simocephalus vetulus	2.7	1 (littoral)	5 (Simoc. type)	S.ve

3. Selection of orthogonal eigenvectors describing β-diversity patterns

For β-diversity, the pair-wise output values obtained from Bray-Curtis and COMDIST (see Material & Methods) were synthesized into Principal Coordinate Analysis (PCoA) eigenvectors after Lingoes correction (Borcard et al. 2011; Swenson 2014). In principle, all eigenvectors generated by PCoA could be used as descriptors of β-diversity patterns in subsequent analysis. However, using all of them can introduce confounding effects in the analyses (Anderson & Willis 2003). Since each eigenvector is an orthogonal synthetic variable representing gradients in β-diversity patterns, it is possible that some

of these gradients are unexplained by the measured factors, which might introduce confounding effects in posterior analyses. A solution is to select a subset of orthogonal eigenvectors that maximizes the association between patterns of β-diversity (taxonomic or functional-phylogenetic) and a set of explanatory variables (Anderson & Willis 2003). To select how many orthogonal eigenvectors should be retained for subsequent analysis, we applied an approach that is suitable for direct multiple regression analyses (Anderson & Willis 2003) [see also (Duarte et al. 2012)]. The selection procedure consisted of first including a single eigenvector (i.e., the first eigenvector, which captures most of the variation in the original distance matrix) describing β-diversity patterns as our response variable into the variation partitioning approach. Then, we computed the total $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ obtained for this combination of this single eigenvector (the first) as response variable and the selected environmental and spatial descriptors as explanatory variables. Next, we included the first two orthogonal eigenvectors as response variables and repeated the procedure, computing again the $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ for this combination of the first two eigenvectors and the predictor variables. This incremental approach was applied consecutively by including an increasing number of orthogonal eigenvectors (i.e., 1,2,3,4 and so forth), until we obtained a complete distribution of $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ values for each number of eigenvectors (response variables) included. Finally, we retained as many eigenvectors as required to maximize $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$, which is the exact number that represents the best fit between the response and the explanatory matrices. In other words, when including less than that specific number of eigenvectors this

results in a lower $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ because it captures too low variation in the original response matrix. In contrast, including more than that specific number decreases the $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ by adding residuals associated to redundant, meaningless variables [for further details on this selection procedure see also Anderson and Willis (2003) and Duarte et al. (2012)].

4. Results of a taxonomic approach based on Bray-Curtis

Using a taxonomic index of beta-diversity (i.e., Bray-Curtis) followed by PCoA analysis, resulted in a moderate increase of explanatory power provided by environmental and spatial factors compared to a taxonomic approach based only on species abundances after heelinger transformation. However, the conclusions that can be drawn from both taxonomic-based approaches are largely the same. Specifically, the amount of explanatory power provided by environmental, urbanization and spatial factors are much higher for trait and phylogenetic-based approaches than for any of the taxonomic-based approaches used (Fig. S2). Variation partitioning revealed that the relative importance of local environmental factors was much more important than that of spatial factors (Fig. S.2). Finally, using the taxonomic approach based on Bray-Curtis resulted in a lack of explanatory power provided by urbanization (i.e., no urbanization variable was selected through the forward selection procedure approach).

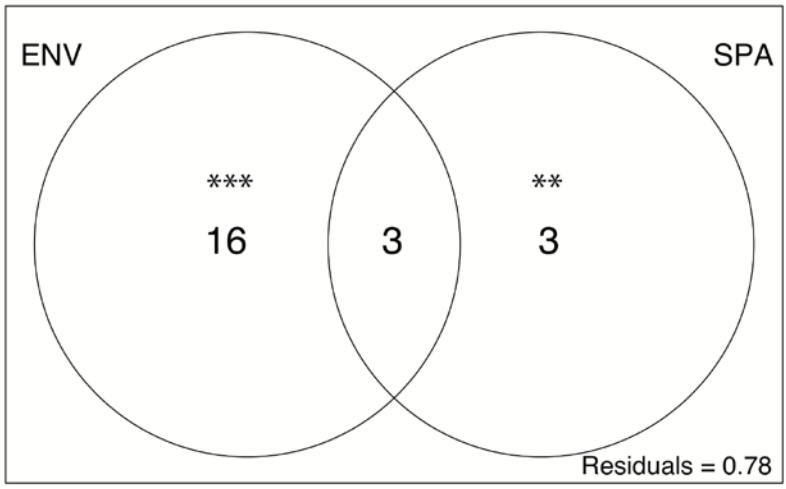

Figure S2: Venn diagram depicting the results of variation partitioning (i.e., explained variation; % $_{adj}R^2$) for metacommunity analysis based on Bray-Curtis approach. Env = environmental model; Spa = spatial model. Three asterisks represent significant results $p < 0.001$; two asterisks $p < 0.01$.

5. Correlation between body size, phylogeny and the urbanization gradient

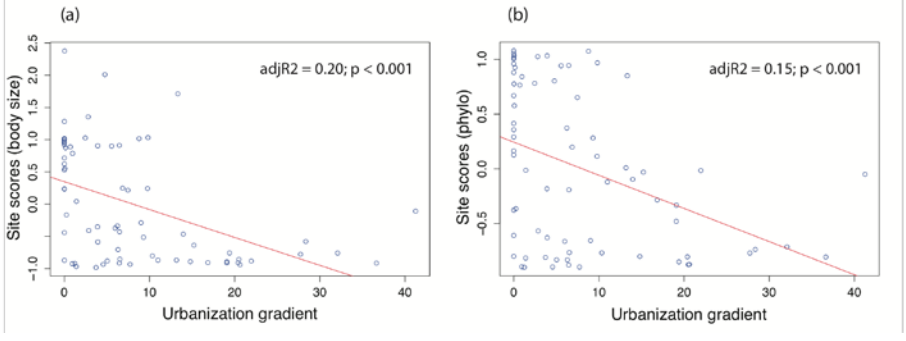

Figure S3: Correlation between urbanization (percentage built-up area at 50 meters) and body size composition within ponds (a) and

between urbanization and phylogenetic composition within ponds (b). On the Y-axis site scores obtained from the first axis of PCoA based on COMDIST for body size (a) and phylogenetic distances (b) are given. (a) More positive scores are associated with communities dominated by larger species whereas negative scores are associated with communities dominated by smaller species. (b) More positive scores are associated with communities dominated by Daphiniidae species whereas more negative scores are associated with communities dominated by Chydoriidae species.

6. Analysis of residuals

We observed a large amount of unexplained variance when using a taxonomic-based approach (i.e., traditional metacommunity analysis based on species abundances after hellinger transformation). This large fraction of unexplained variation could be due to idiosyncratic responses of species with similar traits. This can happen for example, when different species with similar traits respond similarly to the same type of environmental, urbanization or spatial factors. To test this idea, we first extracted the residuals of the the best performing RDA model using environmental, spatial and urban-related variables on species composition. Then, we ran another variation partiooning analysis as described in the main text, but now using the residuals as response variables and trait (both body size alone or several traits) and phylogenetic information as explanatory variables. We found that trait and phylogenetic information significantly explained variation in the residuals of the taxonomic analysis (Fig. S4). This indicates that using trait and phylogenetic information helps to explain the large amount of

unexplained variation resulting from taxonomic-based approaches. This suggests that closely related species, or species that share similar traits, respond in the same way to environmental, spatial and urbanization gradients, but such species responses cannot be easily predicted by species (names) identities alone.

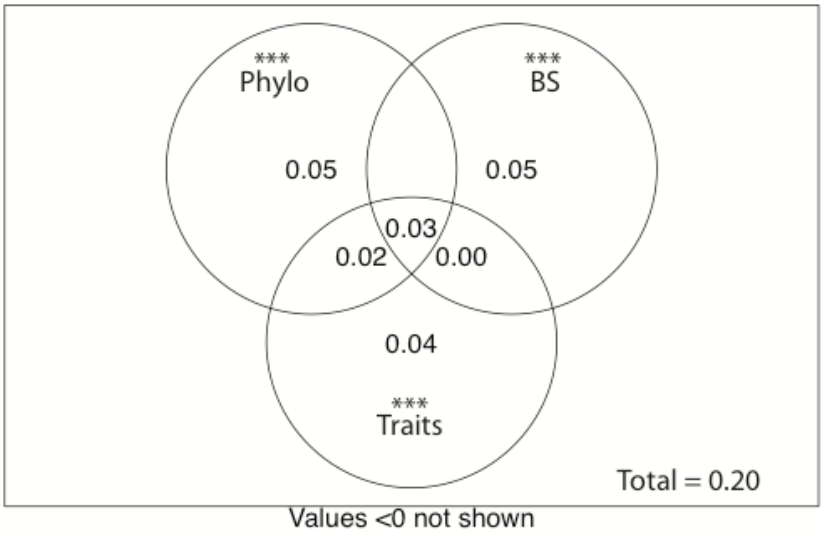

Figure S4. Venn diagrams showing results of variation partitioning using as response variable the residuals of RDA based on selected environmental, spatial and urban-related variables on species composition and as predictors phylogeny (Phylo), body size (BS) and multi-trait metrics (Traits). Three asterisks indicate significant results ($p < 0.001$).

SUPPLEMENTARY INFORMATION TO CHAPTER III

1. Detailed description of the field sampling and sample characterization protocols.

In July-September 2003, 99 ponds were sampled for a number of physical and chemical water parameters and cladoceran zooplankton communities. Three neighboring ponds were sampled per sampling day. To uncouple sampling time from geographic location, thirty-three groups of three neighboring ponds were selected prior to the sampling campaign and the sampling sequence of these groups was randomized. In each pond, pH, conductivity, temperature, alkalinity and water transparency were measured in situ as described in De Bie et al. (2012). The percentage cover of the total macrophyte vegetation as well as of the submerged, floating and emergent macrophytes separately was estimated. For the chemical analysis of water quality, a tube sampler (length 1.5 m; diameter 75 mm) was used to collect a depth-integrated sample of pond water in the open water part of each pond. For zooplankton, 6-L water samples were collected using a tube sampler at 8 different locations in the pond, according to a predefined grid (4 samples in the littoral and 4 samples in the open water area). The 8 samples were pooled and samples for crustacean zooplankton were taken by filtering 40 L through a 64-μm conical net, after which the zooplankton samples were stored on formaldehyde. Fish abundances were assessed by applying point abundance sampling with electrofishing. The anode was immersed in each pond at eight randomly chosen locations and fish were collected with a hand net.

Water samples were analyzed for the concentration of chlorophyll a, nutrients (total phosphorus and nitrates), alkalinity and some major ions (calcium, chloride and sulphate ions, water hardness). Chlorophyll a concentrations were determined spectrophotometrically. Total phosphorus concentration was measured with the ascorbic acid method after perchlorate digestion (Murphy & Riley 1962). Nitrate concentration was determined in GF/F filtered water samples with a Technicon autoanalyser III. Concentrations of sulphates, chlorides, calcium, and alkalinity and hardness were measured following standard methods according to the Hach Water Analysis Handbook (Hach 1992).

2. Molecular-phylogenetic tree reconstruction

We built a molecular-phylogenetic tree for the 35 cladoceran species occurring in the selected metacommunity (91 ponds) according to a recently developed protocol (Roquet et al. 2013). Information on four molecular markers (COI, and 16S, 18S and 28S rDNA) was extracted from Genbank for the 35 species using the browser “Geneious”. This was also done for Sida crystallina, which was not present in the metacommunity but was included as an out-group since it is hierarchically ancestral to all the other cladoceran species included in our study (Braband et al. 2002). The sequences were aligned using the EMBL-EBI web-server (http://www.ebi.ac.uk/Tools/webservices/) under six different alignment models (Clustal omega, Clustal W2, Kalign, MAFFT, MUSCLE and PRANK). The quality of the aligned output files was checked in Bioedit. Per genetic marker, the best alignment was chosen using MUMSA (Lassmann & Sonnhammer

2006) (http://msa.sbc.su.se/cgi-bin/msa.cgi). Since all alignments had an AOS score (average overlap score) above 0.5, we used the highest MOS (multiple overlap score) to select the best alignment model for each molecular marker. After selecting the best alignments, the aligned sequences were trimmed using the automated 1 algorithm in the online software Phylemon2 (Sanchez et al. 2011) (http://phylemon2.bioinfo.cipf.es/). Afterwards a single, concatenated supermatrix with the aligned sequences of all four markers was constructed. For eight species present in our data set no molecular information was yet available in Genbank, and we replaced them by their sister species following recommendations in Helmus et al. (2010) and (Cadotte 2013) (Table S1). Based on literature (Table S2) a constraint tree was constructed [for a similar example of the usage of constraint trees for freshwater zooplankton phylogeny reconstruction see (Helmus et al. 2010)]. This constraint tree is used as the backbone of the phylogeny and constrains the deeper nodes of the tree according to previous information. This allows us to assess species relationships within uncontested groups of species and to estimate branch lengths based on molecular information contained in our supermatrix. The constrained nodes are indicated in Fig. S1. Maximum Likelihood (ML) tree reconstruction and bootstrapping was performed using RAxML (thorough ML searches and rapid bootstrapping algorithm) (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis 2006). Finally, an ultrametric tree was constructed using the Penalized Likelihood method (Kim & Sanderson 2008) by applying the function chronos in the package **ape** in R (Reference R).

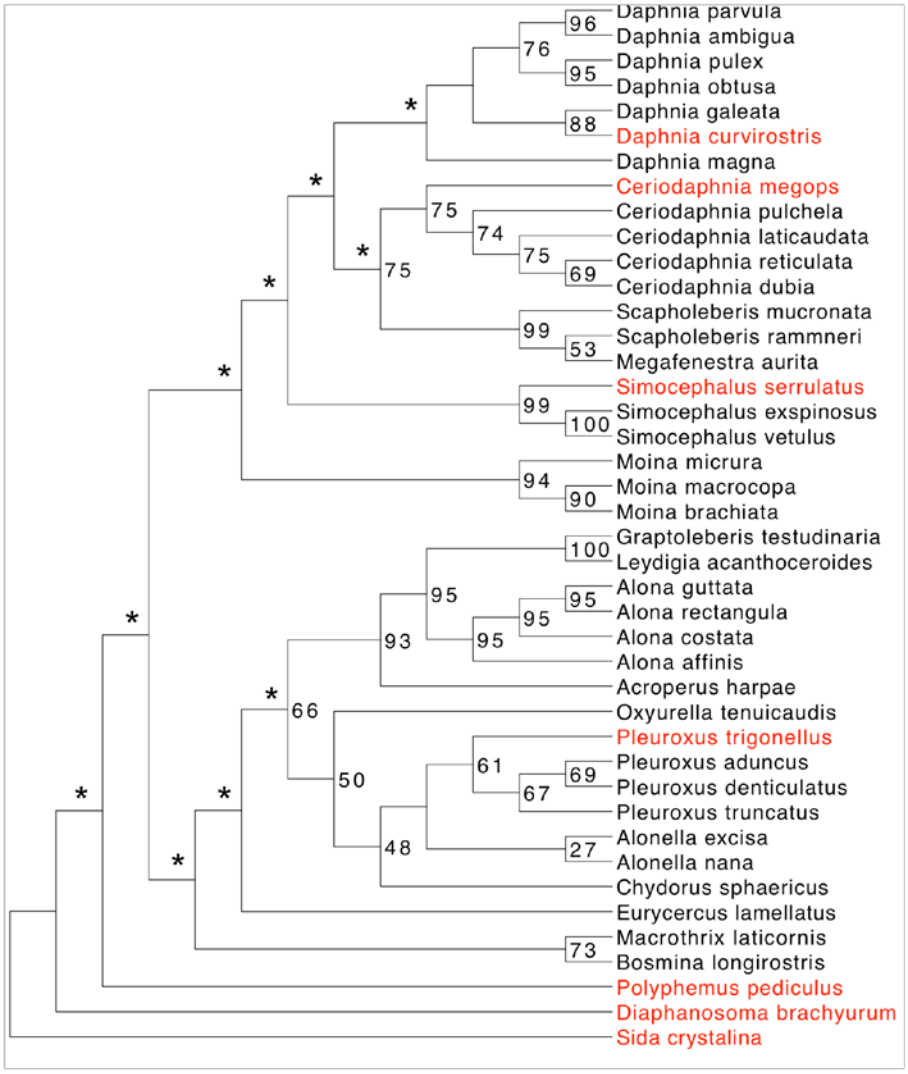

Figure S1. Best-scoring molecular-phylogenetic tree (Maximum Likelihood) showing the evolutionary relationships among 35 cladoceran species recorded in the sampled metacommunity. Bootstrap values are given on the nodes (except for constrained nodes with supporting values lower than 50). Asterisks indicate which nodes were constrained based on previous expert knowledge (Table S2). In red are species that were not present in our dataset but were included

in the phylogenetic tree reconstruction because they are often used in experiments in our group. Those species were dropped from the tree before calculating phylogenetic indices.

Table S4. Species present in our database for which no molecular information was available in Genbank and the species with molecular information that were used to replace the first ones. Note that these are not the same species as marked in red in the phylogenetic tree (those were not in our data-set while the ones in the table below were in our data-set but had no information in Genbank).

Species in our dataset without information in Genbank	**Species with information on Genbank used as representative species**
Alona guttata	*Alona glabra*
Alona rectangula	*Alona pectinata*
Alona costata	*Alona setulosa*
Ceriodaphnia megops	*Ceriodaphnia cornuta*
Alonella nana	*Alonella exigua*
Leydigia acantoceroides	*Leydigia lousi*
Megafenestra aurita	*Scapholeberis armata*
Pleuroxus trigonelus	*Pleuroxus procurvus*

Table S5. References used to constrain deep nodes of the phylogeny and thus establish the main relationships among clades.

Family	**References**

Daphnidae	Adamowicz et al. 2009
Moinidae	Braband et al. 2002
Chydoridae	Sacherová & Hebert 2003
Eurycercidae;	Braband et al. 2002; Helmus et al. 2010
Bosminidae;	
Polyphemus sp.; Sididae	

3. Trait information

We extracted information on body size and plant association from the literature. Body size values are according to Alonso (1996). Values from the trait plant association were determined mainly based on Barnett et al. (2007) and complemented with information provided by Declerck et al. (2007) and Alonso (1996). Trait values are given in Table S3.

Table S6: List of species recorded in out study and their respective trait values.

Species	Body size (mm)	Plant association
Acroperus harpae	0.8	Littoral (3)
Alona affinis	1	Littoral (3)
Alonella excisa	0.4	Littoral (3)
Alonella exigua	0.35	Littoral (3)
Alona glabra	0.26	Littoral (3)
Alona pectinata	0.4	Littoral (3)
Alona setulosa	0.5	Littoral (3)
Bosmina longirostris	0.6	Pelagic (1)

Species	Body size (mm)	Plant association
Ceriodaphnia dubia	1.4	Intermediate (2)
Ceriodaphnia laticaudata	0.9	Intermediate
Ceriodaphnia pulchela	0.8	Intermediate (2)
Ceriodaphnia reticulata	1.3	Intermediate (2)
Chydorus sphaericus	0.5	Intermediate (2)
Daphnia ambigua	1.3	Pelagic (1)
Daphnia galeata	2	Pelagic (1)
Daphnia magna	4	Pelagic (1)
Daphnia obtusa	2.5	Pelagic (1)
Daphnia parvula	1.3	Pelagic (1)
Dapnia pulex	2.5	Pelagic (1)
Eyrycercus lamellatus	3.3	Littoral (3)
Graptoleberis testudinaria	0.6	Littoral (3)
Leydigia louisi	0.8	Littoral (3)
Macrothrix laticornis	0.6	Littoral (3)
Moina brachiata	1.6	Pelagic (1)
Moina macrocopa	1.5	Pelagic (1)
Moina micrura	1.2	Pelagic (1)
Oxyurella longirostris	0.6	Littoral (3)
Pleuroxus aduncus	0.65	Littoral (3)
Pleuroxus denticulatus	0.6	Littoral (3)
Pleuroxus truncatus	0.65	Littoral (3)
Megafenestra aurita	1.6	Intermediate (2)
Scapholeberis mucronata	1.2	Intermediate (2)
Scapholeberis rammneri	1.35	Intermediate (2)
Simocephalus expinosus	2.8	Littoral (3)

Species	Body size (mm)	Plant association
Simocephalus vetulus	2.7	Littoral (3)

4. Selection of response variables: orthogonal eigenvectors describing β-diversity patterns

For β-diversity, the pair-wise output values obtained from Bray-Curtis and COMDIST (see Material & Methods) were synthesized into Principal Coordinate Analysis (PCoA) eigenvectors after Lingoes correction (Borcard et al. 2011; Swenson 2014). In principle, all eigenvectors generated by PCoA could be used as descriptors of β-diversity patterns in subsequent analyses. However, using all of them might introduce confounding effects in the analyses (Anderson & Willis 2003). Since each eigenvector is an orthogonal synthetic variable representing gradients in β-diversity patterns, it is possible that some of these gradients are unexplained by the measured factors, which might introduce confounding effects in posterior analyses. A solution is to select a subset of orthogonal eigenvectors that maximizes the association between patterns of β-diversity (taxonomic or functional-phylogenetic) and a set of explanatory variables (Anderson & Willis 2003). To select how many orthogonal eigenvectors should be retained for subsequent analysis, we applied an approach that is suitable for direct multiple regression analyses (Anderson & Willis 2003) [see also (Duarte et al. 2012)]. The selection procedure consisted of first including a single eigenvector (i.e., the first eigenvector, which captures most of the variation in the original distance matrix) describing β-diversity patterns as our response variable into the variation partitioning approach. Then, we

computed the total $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ obtained for this combination of this single eigenvector (the first) as response variable and the selected environmental and spatial descriptors as explanatory variables. Next, we included the first two orthogonal eigenvectors as response variables and repeated the procedure, computing again the $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ for this combination of the first two eigenvectors and the predictor variables. This incremental approach was applied consecutively by including an increasing number of orthogonal eigenvectors (i.e., 1,2,3,4 and so forth), until we obtained a complete distribution of $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ values for each number of eigenvectors (response variables) included. Finally, we retained as many eigenvectors as needed to maximize $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$, which is the exact number that represents the best fit between the response and the explanatory matrices. In other words, when including less than that specific number of eigenvectors this results in a lower $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ because it captures too low variation in the original response matrix. In contrast, including more than that specific number decreases the $_{adj}R^2_{(\mathbf{Y}|\mathbf{X})}$ by adding residuals associated to redundant, meaningless variables [for further details on this selection procedure see also Anderson and Willis (2003) and Duarte et al. (2012)].

5. Exploring the drivers of taxonomic richness and evenness

Similarly to patterns reported in the main text for the exponential of Shannon diversity index (i.e., Shannon entropy; Jost 2006), no environmental or spatial variables were selected in the forward selection procedure (Blanchet et. al 2008) as significant drivers of taxonomic species richness or evenness (i.e., Pielou J evenness index).

Additionally, there was a strong correlation between Shannon entropy and species richness ($_{adj}R^2$ = 0.445, p < 0.001) as well as between Shannon entropy and evenness ($_{adj}R^2$ = 0.527, p < 0.001). Therefore, the information provided by these three taxonomic diversity metrics were redundant and uninformative in our study case and we present in the main text only results obtained for Shannon entropy, because it is a widely used taxonomic diversity metric and because it is more comparable with our abundance-weighed functional-phylogenetic *a*-diversity metrics.

6. R-packages used for specific applications.

Table S4: List of the main R-packages used for specific applications.

R-package	**Application**
PCNM	Used to generate spatial descriptors
packfor	Forward selection with permutation
ape	Penalized Likelihood method for phylogenetic tree reconstruction
picante	Trait-phylogenetic analyses

6. References in the Appendix.

Adamowicz, S.J., Petrusek, A., Colbourne, J.K., Hebert, P.D.N. & Witt, J.D.S. (2009). The scale of divergence: A phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. Molecular phylogenetics and evolution, 50, 423-436.

Alonso, M. (1996). Fauna Iberica. Vol. 7, Crustacea, Branchiopoda.- Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Cientificas, Madrid, Spain. Pp. 1-486. Journal of Crustacean Biology, 17, 758-759.

Anderson, M.J. & Willis, T.J. (2003). Canonical Analysis of Principal Coordinates: A Useful Method of Constrained Ordination for Ecology. Ecology, 84, 511-525.

Barnett, A.J., Finlay, K. & Beisner, B.E. (2007). Functional diversity of crustacean zooplankton communities: towards a trait-based classification. Freshwater Biology, 52, 796-813.

Blanchet, F. G. et al. 2008. Forward Selection of Explanatory Variables. — Ecology 89: 2623-2632.

Borcard, D., Gillet, F. & Legendre, P. (2011). Numerical Ecology with R. Springer New York Dordrecht London Heidelberg.

Braband, A., Richter, S., Hiesel, R. & Scholtz, G. (2002). Phylogenetic relationships within the Phyllopoda (Crustacea, Branchiopoda) based on mitochondrial and nuclear markers. Molecular phylogenetics and evolution, 25, 229-244.

Cadotte, M.W. (2013). Experimental evidence that evolutionarily diverse assemblages result in higher productivity. Proceedings of the National Academy of Sciences of the United States of America, 110, 8996-9000.

De Bie, T., De Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D. et al. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. Ecology letters, 15, 740-747.

Declerck, S., Vanderstukken, M., Pals, A., Muylaert, K. & Meester, L.D. (2007). Plankton Biodiversity Along a Gradient of Productivity and Its Mediation by Macrophytes. Ecology, 88, 2199-2210.

Duarte, L.D.S., Prieto, P.V. & Pillar, V.D. (2012). Assessing spatial and environmental drivers of phylogenetic structure in BrazilianAraucariaforests. Ecography, 35, 952-960.

Jost, L. 2006. Entropy and diversity. — Oikos 113: 363-375.

Hach (1992). Hach Water Analysis Handbook. Hach Company.

Helmus, M.R., Keller, W., Paterson, M.J., Yan, N.D., Cannon, C.H. & Rusak, J.A. (2010). Communities contain closely related species during ecosystem disturbance. Ecology letters, 13, 162-174.

Kim, J. & Sanderson, M.J. (2008). Penalized likelihood phylogenetic inference: bridging the parsimony-likelihood gap. Syst Biol, 57, 665-674.

Lassmann, T. & Sonnhammer, E.L.L. (2006). Kalign, Kalignvu and Mumsa: web servers for multiple sequence alignment. Nucleic Acids Research, 34, W596-W599.

Murphy, J. & Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27, 31-36.

Roquet, C., Thuiller, W. & Lavergne, S. (2013). Building megaphylogenies for macroecology: taking up the challenge. Ecography, 36, 13-26.

Sacherová, V. & Hebert, P.D.N. (2003). The evolutionary history of the Chydoridae (Crustacea: Cladocera). Biological Journal of the Linnean Society, 79, 629-643.

Sanchez, R., Serra, F., Tarraga, J., Medina, I., Carbonell, J., Pulido, L. et al. (2011). Phylemon 2.0: a suite of web-tools for molecular evolution, phylogenetics, phylogenomics and hypotheses testing. Nucleic Acids Research, 39, W470-W474.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22, 2688-2690.

Swenson, N.G. (2014). Functional Phylogenetic Ecology in R. Springer New York Heidelberg Dordrecht London.

SUPPORTING INFORMATION APPENDIX S2

SUPPORTING INFORMATION APPENDIX S3

SUPPORTING INFORMATION APPENDIX S4

Series of extensive tables showing detailed results for integrated trait and phylogenetic analyses of alpha and beta diversity. These appendices are permanently deposited online and can be accessed electronically using the following link: http://onlinelibrary.wiley.com/doi/10.1111/ecog.02263/full

SUPPLEMENTARY INFORMATION TO CHAPTER IV

Details on methods and supplementary results

1. Molecular-phylogenetic tree reconstruction and phylogenetic signal in body size

We created a molecular phylogenetic tree for 42 cladoceran species previously recorded in Belgium (De Bie et al. 2012) following a recently developed protocol (Roquet et al. 2013). To do this, we extracted information from Genbank on four molecular markers (COI, and 16S, 18S and 28S rDNA) for the 42 species using the browser "Geneious V. R9.1" (available at http://www.geneious.com/download). *Sida crystallina* was included as an out-group because it is hierarchically ancestor to all the other Cladoceran species represented in the phylogenetic tree (Braband et al. 2002). We used the EMBL-EBI web-server (http://www.ebi.ac.uk/Tools/webservices/) to align the sequences based on six different alignment tools (Clustal omega, Clustal W2, Kalign, MAFFT, MUSCLE and PRANK). We then checked the quality of the aligned output files in Bioedit (Hall 1999). The best alignment per genetic marker was chosen using MUMSA (Lassmann and Sonnhammer 2006) (http://msa.sbc.su.se/cgi-bin/msa.cgi). Since all alignments presented an "average overlap score" above 0.5, we used the highest "multiple overlap score" to select the best alignment model for each molecular marker. After selecting the best alignments, the aligned sequences were trimmed using the automated 1 algorithm

in the software Phylemon2 (Sanchez et al. 2011) (http://phylemon2.bioinfo.cipf.es/). Afterwards, we concatenated all the aligned sequences in a supermatrix. Based on the literature (Table S1) a constraint tree was built [see also Helmus et al. (2010) for a similar example of the application of a constraint tree for freshwater zooplankton phylogeny reconstruction]. This constraint tree was used as the backbone of the phylogeny to constrain the deeper nodes of the tree. This allowed us to assess species evolutionary relationships within uncontested groups of species and to estimate branch lengths based on molecular information contained in our supermatrix. The constrained nodes are indicated in Fig. S1. We used the Maximum Likelihood (ML) approach for phylogenetic tree reconstruction and bootstrapping was performed using RAxML (thorough ML searches and rapid bootstrapping algorithm) (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis 2006). Finally, we ultrametricized the tree using the Penalized Likelihood method (Kim and Sanderson 2008) using the function *chronos* in the package **ape** in R (R Core Team 2014). The phylogenetic tree is presented in figure S1 and a reduced phylogenetic tree (containing only the species used in this experiment) can be found in Figure S3.

2. Phylogenetic signal in body size

Using the phylogenetic tree in figure S1 and body size values obtained from the literature, we tested for phylogenetic signal in body size of Cladocera zooplankton (this test was part of another recent study; Gianuca et al. under review). To do this we used the K-statistic

(Blomberg et al. 2003). Obtained K-values were compared to randomized K-values. Significant p-values indicate non-random correspondences between traits and phylogeny. Values of K higher than 1 indicate that traits are more conserved than expected by a Brownian Motion Evolutionary model (Blomberg *et al.* 2003). Values lower than 1 indicate that traits are less conserved along the phylogeny than expected by this model.

We found no evidence for phylogenetic signal in body size for the zooplankton species occurring in this Belgian metacommunity ($K = 0.04$, $p = 0.243$).

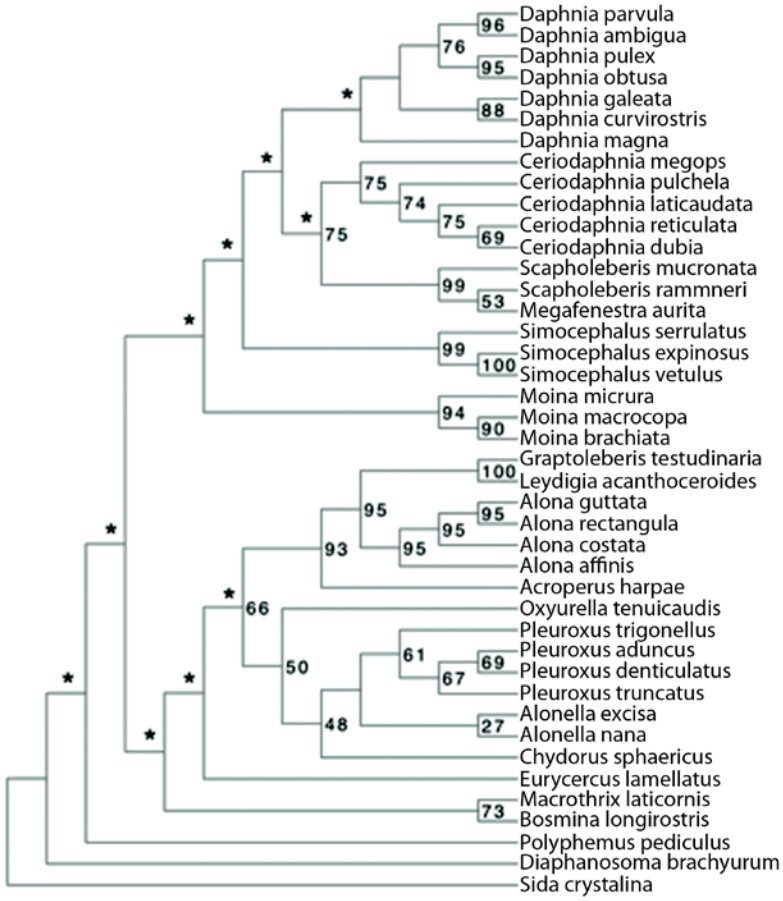

Figure S2: Best-scoring molecular-phylogenetic tree (Maximum Likelihood) showing the evolutionary relationships among 42 cladoceran species previously recorded in Belgium. Bootstrap values are given on the nodes (except for constrained nodes with supporting values lower than 50). Asterisks indicate which nodes were constrained based on previous expert knowledge (Table S1).

Table S7. References used to constrain deep nodes of the phylogeny and thus establish the main relationships among clades.

Family	**References**

Daphnidae	Adamowicz et al. (2009)
Moinidae	Braband et al. (2002)
Chydoridae	Sacherová and Hebert (2003)
Eurycercidae; Bosminidae; Polyphemus sp.; Sididae	Braband et al. (2002); Helmus et al. (2010)

3. **Experimental design and species characteristics**

We choose from the 42 species presented in Appendix S1 a set of eight species that are widespread in Europe [e.g., (Alonso 1996, Louette et al. 2007)], relatively abundant, easy to culture in the laboratory and that largely conform with our conceptual figure 2 presented in the main text. The functional trait (body size) and phylogenetic distances among the species used in the grazing experiments are presented in Figure S2 below. More information on species characteristics can be found in tables S2 and S3.

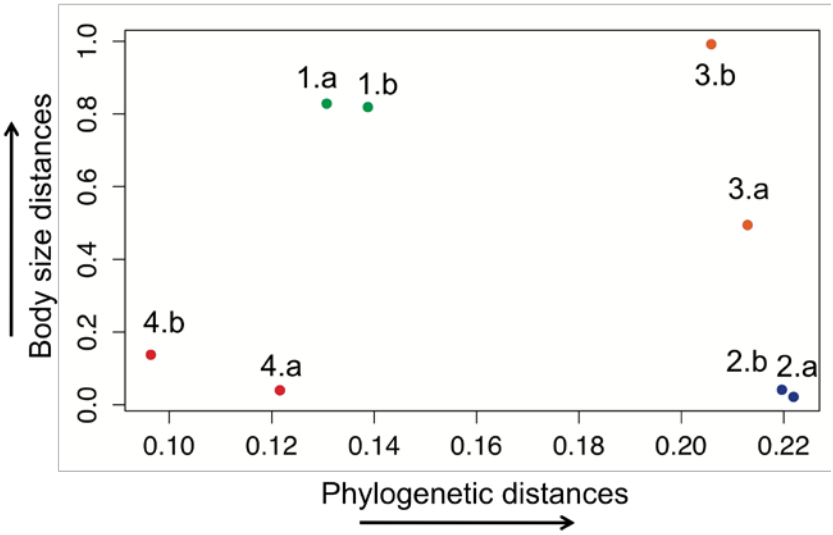

Figure S3. Relationship between body size and phylogenetic distances among species pairs. The functional trait and phylogenetic axes were not significantly correlated to each other ($_{adj}R^2$ = -0.044, p = 0.873). Codes are given in table S2. Treatment (i) is coloured in green and represent closely related species that have diverged in body size; treatment (ii) in blue represent distantly related species that have converged in body size; treatment (iii) in orange represent distantly related species that are dissimilar in body size; and treatment (iv) in red represent closely related species that have similar body sizes. Note that species pair 3.a is comparatively less diverse in size than 3.b. This happened because we choose species combinations based on body size data from the literature, which proved later on not to exactly match our own body size measurements.

Table S2: List of species combinations used in the experiments. Combinations were selected based on the following criteria: (1) HIGH FD - LOW PD, closely related species that differ strongly in body

size; (2) HIGH FD - LOW PD; distantly related species that overlap in body size (cf. convergence); (3) HIGH FD - HIGH PD, distantly related species that differ strongly in body size; (4) LOW FD - LOW PD, closely related species that differ little in body size (cf. scenarios 3 and 4 represent body size conservatism along the phylogeny). For each treatment, there were two species combinations and for each species combination there were three replicates. For more details see methods and figure 2 in the main text. **Please, find Table S2 as online with the following link: http://rspb.royalsocietypublishing.org/content/royprsb/suppl/2016/04/11/rspb.2016.0487.DC1/rspb20160487supp1.pdf**

Table S3. List of species used in the experiments with their corresponding body size and biomass values. In the third column, “N” refers to the number of individuals used in each species combinations. When assessing individual grazing performances, we doubled that number to keep biomass constant among all treatments and replicates.

Species	Body size	Biomass	N
Alona affinis	0.88	3.36	25
Daphnia ambigua	0.94	3.9	22
Daphnia curvirostris	2.01	8.68	10
Daphnia magna	3.6	28.6	3
Daphnia pulex	1.9	8.1	10
Eurycercus lamellatus	1.95	7.96	10
Pleuroxus aduncus	0.52	2.06	35
Pleuroxus truncatus	0.63	2.4	35

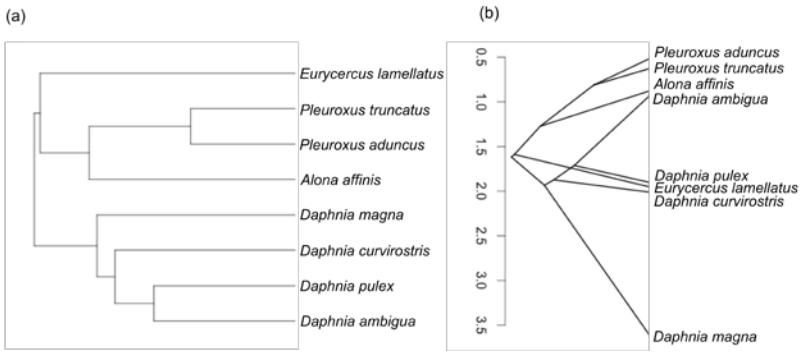

Figure S4: Phylogenetic and trait relationships among the cladoceran species used in this study. (a): the phylogenetic tree based on Maximum Likelihood; (b) the evolutionary-traitgram, which posits the tips of the phylogeny according to a trait axis (here body size in mm) while keeping the internal nodes according to evolutionary distance among species (genetic distance in this example). See Cadotte et al. (2013) for more details on the evolutionary trait-gram.

4. Average body sizes within communities and observed grazing rates

To further test if increasing average body size within communities resulted in higher grazing rates, we use linear regression. In this analysis we used average body size within communities as predictor and grazing rates as response variable. We found a strong positive correlation between average body size within communities and observed grazing rates (Fig. S4) ($_{adj}R^2 = 0.56$; $p < 0.001$).

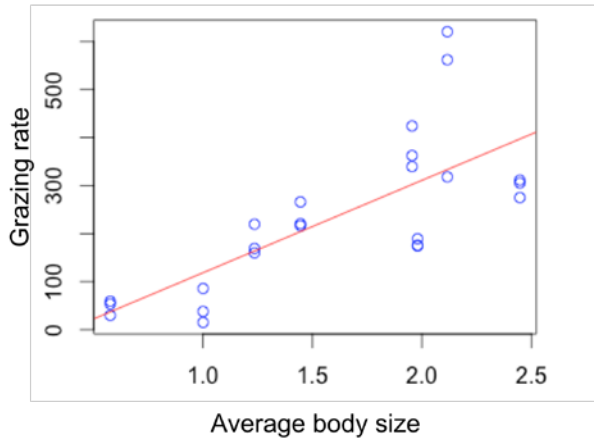

Figure S5. Scatterplot depicting the positive correlation between increasing average community body size and total observed grazing rates.

5. Functional and phylogenetic diversity as predictors of species interactions and its impact on top-down control

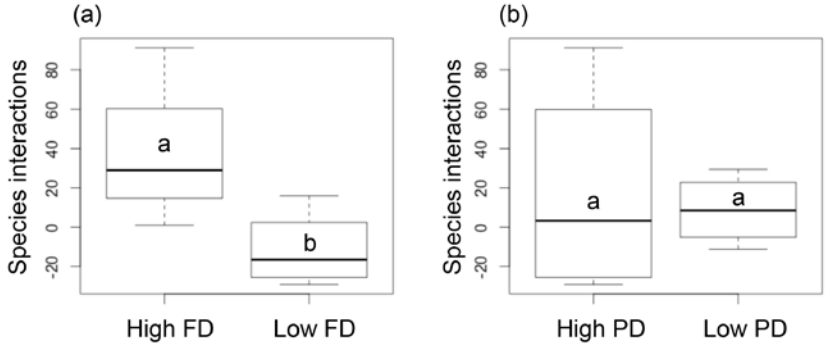

Figure S6. Relative increase or decrease in resource uptake efficiency for every species combination. Positive values refer to positive species interactions; negative values refer to negative species interactions. Zero refers to a situation in which the two-species communities present a grazing impact that is identical to the average of the two

species in monoculture. Box plots depict the effect of functional diversity (FD) (a) and phylogenetic diversity (PD) (b) on species interactions. HIGH FD refers to treatments (i) and (iii), whereas LOW FD refers to treatments (ii) and (iv) in Figure 2. HIGH PD refers to treatments (ii) and (iii) in Figure 2, whereas LOW PD refers to treatments (i) and (iv). Distinct letters (a vs. b) indicate significant differences ($p < 0.001$) based on two-way permutation univariate ANOVA.

6. Variation partitioning analysis for species combinations that present phylogenetic signal in body size

Here we constrained the variation partitioning analysis (see methods in the main text for details) to treatments where species combinations presented size conservatism (i.e., treatments 3 and 4 only). This allowed us to assess the explanatory power provided by phylogeny on top-down control when body size is conserved along the phylogeny.

6.1 Results

The results obtained when considering only experimental treatments designed to reflect trait conservatism indicated that phylogenetic and trait diversity together explained a large proportion of grazing rate ($_{adj}R^2 = 0.287$). In this context, however, neither the pure effect of size diversity nor the pure effect of phylogenetic diversity was significant ($_{adj}R^2 = 0.071$, $p = 0.194$; and $_{adj}R^2 = -0.061$, $p = 0.741$, respectively). This suggests that the predictive power of phylogeny depends entirely on body size conservatism along the phylogeny and that phylogenetic

distances do not reflect any additional information on unmeasured effect traits relevant to this ecosystem function.

7. Size-related grazing efficiency asymmetry or size-related niche differences as the main mechanism mediating top-down control?

The idea of size-related niche differences as the main mechanism affecting top-down control is based on the assumption that smaller zooplankton species are more efficient grazers on smaller phytoplankton while large zooplankton species are more efficient grazers on large phytoplankton (Hansen et al. 1994, Cyr and Curtis 1999, Ye et al. 2013), thus resulting in feeding complementarity. In contrast, the hypothesis of size-related grazing efficiency asymmetry as the main mechanism mediating top-down control is based on the assumption that larger and small zooplankton species overlap in their main food particles, but the former are more efficient grazers over the entire spectrum of phytoplankton cell sizes (Brooks and Dodson 1965, Dodson 1974, Mourelatos and Lacroix 1990).

To test which of these two mechanisms better explain the relationship between body size compositional differences and top-down control, we here complement the results from the ANOVA approach (see main text) using a linear regression approach. The main differences between such approaches (ANOVA and linear regression) is that the former is based on an artificial cut of species groups based on body sizes (i.e., small vs. large) whereas the latter is based on the continuous distributions of average body sizes among species.

Using grazing rate performances of each species in monoculture, we performed linear regressions to test the hypothesis that small zooplankton species are more efficient grazers on small algae cells and that large zooplankton are more efficient grazers on large algae cells. For this analysis, we used body size as a predictor and grazing rates as a response variable using a linear model in the R statistical package (R Core Team 2014). Here we distinguished between (1) total grazing rates (i.e., the entire spectrum of phytoplankton cell sizes); (2) grazing rates on small algae cells; and (3) grazing rates on large algae cells. Results from ANOVA and linear regression approaches were very similar.

7.1 Results

The linear regression analyses revealed that larger zooplankton species depleted both large and small phytoplankton more efficiently than small zooplankton (large algae: $_{adj}R^2 = 0.70$, $p < 0.001$; small algae: $_{adj}R^2 = 0.26$, $p = 0.005$). The larger species were therefore superior grazers over the entire spectrum of algae sizes ($_{adj}R^2 = 0.69$, $p < 0.001$) (Fig. S2c).

8. Quantifying the pure and shared effects of community average size (CAS) and phylogenetic composition on community grazing rates

In a similar way that body size can be separated in two components (i.e., variance and mean), phylogenetic effects on grazing rates can

also be separated in a variance component (i.e., phylogenetic diversity within assemblages) and a compositional component (i.e., lineage composition). For instance, two communities may present a pattern of low phylogenetic diversity (i.e., phylogenetic clustering) but differ a lot in terms of lineage composition; e.g., communities composed only of Daphniidae species and communities composed only of Chydoriidae species. While our study was carefully designed to separate the effects of body size and phylogenetic diversity, it was not possible to design it so as to completely separate the effects of community average size (CAS) from that of phylogenetic composition. This happened due to the inexistence of Chydoriidae species as large as *Daphnia magna* (i.e., our largest species) as well as Daphniidae species as small as *Pleuroxus aduncus* (i.e., our smallest species). In order to quantify the independent and shared contributions of community average size and phylogenetic composition on grazing rates, we used variation partitioning (for more details on the method of variation partitioning, please see methods in the main text). In this case, we used as a response variable the observed grazing rates and as predictors community average size and phylogenetic composition. The variable phylogenetic composition was a categorical variable (1 = Daphniidae; 2 = Daphniidae and Cydoridae; 3 = Chydoridae).

8.1. Results

Variation partitioning revealed that the explanatory variables CAS and phylogenetic composition and their intersections accounted for approximately 56% of observed variation in grazing rates (Fig. S5).

Community average size (CAS) accounted for 100 % of the total amount of explained variation, either as a pure effect (33% of explained variation) or shared with phylogenetic composition (23% of explained variation). The pure effect of CAS was highly significant ($p < 0.001$), whereas the pure effect of phylogenetic composition was not significant ($p = 0.452$).

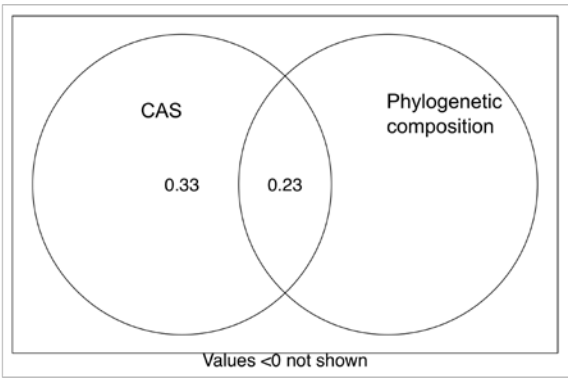

Figure S7: Venn diagrams showing the pure and shared effects of community average size (CAS) and phylogenetic composition on community grazing rates.

SUMMARY

Increasing anthropogenic pressures on earth are leading to faster rates of species extinctions, and this is undermining a variety of ecosystem functions and the provisioning of ecosystem services to humanity. The biodiversity concept is multifaceted and can be quantified at several dimensions and hierarchical spatial scales. We have to better understand the drivers and consequences of biodiversity if we are to properly manage ecosystems in an era of species extinctions. In this PhD research we aimed at quantifying the importance of local and regional processes on biodiversity patterns, to understand how anthropogenic pressures influence different dimensions of biodiversity, and how different biodiversity dimensions scale up to affect a key ecosystem function.

In **chapter I** we partitioned β-diversity into its replacement and nestedness components and used an experimental metacommunity approach to test how environmental heterogeneity associated with a nutrient gradient and dispersal processes affect each component of β-diversity. We clearly demonstrate that in unconnected, heterogeneous landscapes β-diversity is dominated by a nestedness pattern. However, increasing dispersal erased completely the nestedness pattern and resulted in β-diversity derived only from species replacement. This indicates that the nutrient addition treatment results in directional species losses in unconnected landscapes. However, when dispersal is enough, some species are rescued from local extinction, which reduces nestedness. At the same time, dispersal allows species to track favorable environmental conditions at the landscape scale, leading to a

pattern of high replacement via species sorting. We found distinct patterns of nestedness and replacement in environmentally homogeneous landscapes. Particularly, we found higher species replacement in unconnected, homogeneous landscapes whereas increasing dispersal homogenized the metacommunity. This suggests that stochastic drift is a major determinant of regional diversity in unconnected, homogeneous landscapes. The nestedness component remained unchanged across the dispersal gradient in environmentally homogeneous landscapes.

In **chapter II** we used a hierarchical sampling design in which urbanization levels were quantified at seven spatial scales (i.e., ranging from 50m to 3200m radii) in order to assess how urbanization affects the taxonomic, functional and phylogenetic dimensions of zooplankton diversity. We found that urbanization imposes a strong selection pressure on zooplankton species traits and in this way affects phylogenetic composition of the entire metacommunity, but only when urbanization is quantified at the smallest scale considered (i.e., 50m radius). We also show that using species functional traits and/or phylogenetic distances increases significantly the explanatory power provided by environmental, spatial and urbanization processes on β-diversity. This indicates that the processes affecting β-diversity in our study case are mediated by traits and evolutionary relatedness rather than by species identities (i.e., different species with similar traits respond similarly to the sampled gradients). The fact that urbanization effects are significant only at the smallest spatial scale indicates that managing environmental conditions locally has the power to

counteract the effects of regional scale urbanization on biodiversity patterns.

In **chapter III** we propose a conceptual framework for integrating traits and phylogenetic distances to assess scale-dependent community assembly processes. We show that different traits respond in divergent ways to processes operating at different spatial scales and that combining those traits blindly into a single trait-based metric compromises the power of the analysis. We propose that different traits should be classified based on their explanatory power to processes at different spatial scales. We then recommend integrating such traits *a posteriori* with phylogenetic distances to assess the power of unmeasured, phylogenetic conserved traits to complement the information provided by the measured traits. Phylogenetic distances can be used as a benchmark to scale the informative power of specific traits and to assess the potential of phylogeny to capture unmeasured traits. Another key advantage of our approach is that one can assess how evolutionary processes leading to trait conservatism, trait convergence or trait divergence combine to influence current species distributions.

In **chapter IV** we used an experimental approach to disentangle the role of body size and phylogenetic distances to predict a key ecosystem function in aquatic systems, zooplankton top-down control of algae. We show that grazing rates vary strongly among species pairs but this variation can be explained by body size differences among species, and not by phylogenetic distances. This indicates that zooplankton body size is a key trait linking energy and organic matter across trophic levels (i.e., among producers and

consumers) and that phylogeny does not represent variation in additional traits that are relevant for this ecosystem process. Therefore, phylogeny is likely to be informative for predicting zooplankton top-down control of algae **only when body size is strongly conserved along the phylogeny**. In such a case, phylogenetic distances (and body size itself) will provide superior power to explain variation in zooplankton top-down control than measures of taxonomic diversity that disregard trait information. However, when body size varies randomly along the phylogeny, phylogenetic distances are likely to be uninformative for predicting zooplankton top-down control of algae. Therefore, our study warns against a blind use of phylogeny in ecosystem functioning research.

Overall, in this thesis we have demonstrated the importance of considering different dimensions of biodiversity in metacommunity analysis and ecosystem functioning research. Our analyses demonstrate that taxonomic, functional trait and phylogenetic data can provide complementary insights into the drivers of metacommunity assembly and to predict ecosystem processes. Different traits might respond in distinct ways to environmental gradients across spatial scales. The power of phylogeny similarly depends on the spatial and environmental context and on which traits have a phylogenetic signal. Given such complexities, it is nearly impossible to know *a priori* which dimensions of diversity are more informative as well as which dimensions are redundant (i.e., correlated) or complementary. In this thesis, I make a strong case in favour of approaches that either compare different diversity dimensions (Chapter II and IV) or that integrates different dimensions (Chapter III). From a more applied

viewpoint, this thesis suggests that increased anthropogenic pressures on earth, such as those associated with urbanization and land use intensity, are likely to affect different dimensions of zooplankton diversity in combination. Whereas high nutrient inputs may reduce species diversity locally and select for larger zooplankton species, high dispersal rates may enhance diversity locally and regionally via rescue effects and species sorting, respectively. This indicates potential synergistic effects between fragmentation and nutrient inputs on biodiversity, which is likely to happen due to intensification of agricultural land use. Larger zooplankton species tend to be superior grazers that are more efficient in converting food into biomass production. The observation that increasing urbanization potentially selects against larger zooplankton species suggests indirect negative effects of urbanization on ecosystem processes, such as zooplankton top-down control of algae and herbivorous biomass production. Nonetheless, the association between urbanization and functional trait and phylogenetic composition was significant only when urbanization was quantified at the smallest spatial scale (i.e., 50 m radius), suggesting that managing environmental conditions locally may counteract the influences of urbanization on biodiversity.

SAMENVATTING

De stijgende antropogene druk op de aarde leidt tot een versneld uitsterven van soorten. Dit ondermijnt een scala aan ecosysteemdiensten en de voorziening van ecosysteemdiensten voor de mensheid. Het biodiversiteitsconcept heeft vele gezichten en kan gekwantificeerd worden voor verschillende dimensies en op hiërarchische ruimtelijke schalen. Indien we ecosystemen op gepaste wijze willen beheren in dit tijdperk van versneld uitsterven van soorten moeten we de drijfveren en gevolgen van biodiversiteit beter begrijpen. In dit doctoraatsproefschrift beoog ik het belang van lokale en regionale processen voor biodiversiteitspatronen te kwantificeren, te begrijpen hoe antropogene druk verschillende biodiversiteitsdimensies aantast, en te begrijpen hoe verschillende biodiversiteitsdimensies opschalen om een specifieke sleutelecosysteemfunctie te beïnvloeden.

In **hoofdstuk I** partitioneerde ik β-diversiteit in zijn componenten "replacement" (vervanging van soorten) en "nestedness" (genestheid van soorten) en gebruikte ik een experimentele metagemeenschapsbenadering om te testen hoe omgevingsheterogeniteit geassocieerd aan een nutriëntengradiënt en verspreidingsprocessen elk van deze componenten in β-diversiteit beïnvloeden. Ik stelde vast dat in ongeconnecteerde, heterogene landschappen β-diversiteit gedomineerd werd door een patroon van nestedness. Echter, een toenemende dispersie wiste dit patroon van nestedness volledig uit en resulteerde in β-diversiteit die slechts

gebaseerd was op soortenvervanging. Dit wijst erop dat de toevoeging van nutriënten zorgde voor een directioneel verlies in soorten in ongeconnecteerde landschappen. Echter, wanneer dispersie voldoende voorkomt worden sommige soorten van uitsterven gered, hetgeen nestedness vermindert. Terzelfdertijd laat dispersie soorten toe om geschikte omgevingscondities te volgen op landschapsschaal, hetgeen leidt tot een patroon van sterke soortenvervanging via “species sorting”. Ik vond uitgesproken patronen van nestedness en soortenvervanging in qua omgeving homogene landschappen. Specifiek vond ik hogere soortenvervanging in ongeconnecteerde, homogene landschappen terwijl toenemende dispersie de metagemeenschap homogeniseerde. Dit suggereert dat stochastische drift een belangrijke bepaler is van de regionale diversiteit in ongeconnecteerde, homogene landschappen. De nestedness component bleef ongewijzigd overheen een dispersiegradiënt in qua omgeving homogene landschappen.

In **hoofdstuk II** gebruikte ik een hiërarchisch bemonsteringsplan in dewelke urbanisatiegraad gekwantificeerd werd op zeven ruimtelijke schalen (variërend tussen 50m en 3200m radii) om na te gaan hoe urbanisatiegraad de taxonomische, functionele en fylogenetische dimensies van zoöplanktondiversiteit beïnvloedt. Ik vond dat urbanisatiegraad een sterke selectiedruk oplegde aan zoöplanktonsoortskenmerken, maar dit enkel wanneer de urbanisatiegraad gekwantificeerd werd op de kleinst beschouwde ruimtelijke schaal (50m radius). Ik toonde ook aan dat het gebruik van functionele kenmerken van soorten en/of fylogenetische afstanden

tussen soorten de verklarende kracht verhoogde van omgevings-, ruimtelijke en urbanisatiegerelateerde processen op β-diversiteit. Dit wijst erop dat de processen die β-diversiteit beïnvloeden in onze study case gemedieerd worden door kenmerken en evolutionaire verwantschap eerder dan door soortenindentiteit (i.e. soorten met gelijkaardige kenmerken reageren gelijkaardig op de bemonsterde gradiënt). Het feit dat de effecten van urbanisatiegraad op biodiversiteitspatronen enkel significant zijn op de kleinste ruimtelijke schaal wijst erop dat het beheer van omgevingscondities op lokale schaal de effecten van urbanisatie op regionale schaal kan tegenwerken.

In **hoofdstuk III** stel ik een conceptueel kader voor om kenmerken en fylogenetische afstanden te integreren om schaalafhankelijke gemeenschapsprocessen te onderzoeken. Ik toon aan dat verschillende kenmerken op verscheidene manieren reageren op processen die werkzaam zijn op verschillende ruimtelijke schalen en dat het blind combineren van kenmerken in een enkel kenmerkgebaseerde metriek de sterkte van de analyse compromitteert. Ik stel voor dat verschillende kenmerken geklasseerd zouden moeten worden op basis van hun verklarende kracht in processen op verschillende ruimtelijke schalen. Ik beveel dan aan om dergelijke kenmerken *a posteriori* te integreren met fylogenetische afstanden om de sterkte na te gaan van de niet-opgemeten, fylogenetisch geconserveerde kenmerken als complement van de informatie voorzien door de opgemeten kenmerken. Fylogenetische kenmerken kunnen gebruikt worden als criterium om de informatieve kracht van specifieke kenmerken te

schalen en om het potentieel na te gaan van fylogenetische informatie in het omvatten van niet-opgemeten kenmerken. Een ander belangrijk voordeel van deze benadering is dat men kan nagaan hoe evolutionaire processen leidend tot kenmerkconservatisme, kenmerkconvergentie of kenmerkdivergentie samenwerken om de huidige soortendistributie te beïnvloeden.

In **hoofdstuk IV** gebruikte ik een experimentele benadering om de rol te ontrafelen van lichaamsgrootte en fylogenetische afstand om een sleutelecosysteemfunctie in aquatische systemen te voorspellen, namelijk zoöplankton top-down controle van algengroei. Ik toon aan dat graassnelheden sterk verschillen tussen soortenparen, maar dat deze variatie verklaard kan worden door verschillen in lichaamsgrootte tussen de soorten en niet door fylogenetische afstanden. Dit wijst erop dat zoöplankton lichaamsgrootte een sleutelkenmerk is dat energie en organische materie linkt overheen trofische niveaus (tussen consumenten en producenten) en dat fylogenetische informatie niet representatief is voor bijkomende kenmerken die relevant zijn voor dit ecosysteemproces. Daarom is fylogenetische informatie waarschijnlijk informatief voor het voorspellen van zoöplankton top-down controle van algen **enkel wanneer lichaamsgrootte sterk geconserveerd is overheen de fylogenie**. In een dergelijk geval zal fylogenetische afstand (en lichaamsgrootte zelf) een sterkere invloed verschaffen tot het verklaren van variatie in zoöplankton top-down controle dan metingen van taxonomische diversiteit die deze kenmerkinformatie veronachtzaamd. Echter, wanneer lichaamsgrootte op een random

wijze varieert over de fylogenie zullen fylogenetische afstanden niet informatief zijn om de zoöplankton top-down controle van algen te voorspellen. Mijn studie waarschuwt dus tegen het blind gebruik van fylogenetische informatie in onderzoek naar ecosysteemfuncties.

Algeheel toon ik aan dat nutriëntadditie de zoöplanktondiversiteit lokaal reduceert, terwijl dispersie de diversiteit lokaal (en ook globaal in heterogene landschappen) doet toenemen. Ik toon ook aan dat urbanisatiegraad als een filter werkt en selecteert tegen grote zoöplanktonsoorten. Dit kan problematisch zijn aangezien grotere soorten aanzien worden als superieure grazers van fytoplankton en de sleutel kunnen zijn in het controleren van algenbloeien in stadsvijvers. Niettegenstaande toon ik ook aan dat de effecten van urbanisatiegraad op de verspreiding van zoöplankton enkel op de kleinst beschouwde ruimtelijke schaal werkzaam is (50m radius). Daarom lijkt het lokaal beheer van omgevingscondities voldoende om de effecten van urbanisatiegraad op de verspreiding van functionele kenmerken van zoöplanktonsoorten tegen te werken. Uiteindelijke toon ik het nut aan van het vergelijken en combineren van verschillende dimensies van biodiversiteit om ten volle de drijfveren en gevolgen te begrijpen van biodiversiteitswijzigingen.

REFFERENCES

Ackerly, D. D., and W. K. Cornwell. 2007. A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecol Lett **10**:135-145.

Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche Evolution and Adaptive Radiation: Testing the Order of Trait Divergence. Ecology **87**:S50-S61.

Adamowicz, S. J., A. Petrusek, J. K. Colbourne, P. D. N. Hebert, and J. D. S. Witt. 2009. The scale of divergence: A phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus. Mol Phylogenet Evol **50**:423-436.

Alberti, M., C. Correa, J. M. Marzluff, A. P. Hendry, E. P. Palkovacs, K. M. Gotanda, V. M. Hunt, T. M. Apgar, and Y. Zhou. 2017. Global urban signatures of phenotypic change in animal and plant populations. Proceedings of the National Academy of Sciences:201606034.

Allan, E., P. Manning, F. Alt, J. Binkenstein, S. Blaser, N. Blüthgen, S. Böhm, F. Grassein, N. Hölzel, V. H. Klaus, T. Kleinebecker, E. K. Morris, Y. Oelmann, D. Prati, S. C. Renner, M. C. Rillig, M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, E. Solly, E. Sorkau, J. Steckel, I. Steffen-Dewenter, B. Stempfhuber, M. Tschapka, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, M. Fischer, and J. Knops. 2015. Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. Ecol Lett:n/a-n/a.

Alonso, M. 1996. Fauna Iberica. Vol. 7, Crustacea, Branchiopoda.- Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Cientificas, Madrid, Spain. Pp. 1-486. Journal of Crustacean Biology **17**:758-759.

Altermatt, F., E. A. Fronhofer, A. Garnier, A. Giometto, F. Hammes, J. Klecka, D. Legrand, E. Mächler, T. M. Massie, F. Pennekamp, M. Plebani, M. Pontarp, N. Schtickzelle, V. Thuillier, O. L. Petchey, and M. Spencer. 2015. Big answers from small worlds: a user's guide for protist microcosms as a

model system in ecology and evolution. Methods in Ecology and Evolution **6**:218-231.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626-639.

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecol Lett **9**:683-693.

Anderson, M. J., and D. C. I. Walsh. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs **83**:557-574.

Anderson, M. J., and T. J. Willis. 2003. Canonical Analysis of Principal Coordinates: A Useful Method of Constrained Ordination for Ecology. Ecology **84**:511-525.

Angeler, D. G. 2013. Revealing a conservation challenge through partitioned long-term beta diversity: increasing turnover and decreasing nestedness of boreal lake metacommunities. Diversity and Distributions **19**:772-781.

Arnfield, A. J. 2003. Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat island. International Journal of Climatology **23**:1-26.

Baraloto, C., O. J. Hardy, C. E. T. Paine, K. G. Dexter, C. Cruaud, L. T. Dunning, M.-A. Gonzalez, J.-F. Molino, D. Sabatier, V. Savolainen, and J. Chave. 2012. Using functional traits and phylogenetic trees to examine the assembly of tropical tree communities. Journal of Ecology **100**:690-701.

Barnett, A. J., K. Finlay, and B. E. Beisner. 2007. Functional diversity of crustacean zooplankton communities: towards a trait-based classification. Freshwater Biology **52**:796-813.

Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography **19**:134-143.

Baselga, A. 2012. The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. Global Ecology and Biogeography **21**:1223-1232.

Baselga, A. 2013. Separating the two components of abundance-based dissimilarity: balanced changes in abundance vs. abundance gradients. Methods in Ecology and Evolution **4**:552-557.

Baselga, A., and F. Leprieur. 2015. Comparing methods to separate components of beta diversity. Methods in Ecology and Evolution **6**:1069-1079.

Baselga, A., and C. D. L. Orme. 2012. betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution **3**:808-812.

Bello, F. d., S. Lavorel, S. Lavergne, C. H. Albert, I. Boulangeat, F. Mazel, and W. Thuiller. 2013. Hierarchical effects of environmental filters on the functional structure of plant communities: a case study in the French Alps. Ecography **36**:393-402.

Bender, M. G., F. Leprieur, D. Mouillot, M. Kulbicki, V. Parravicini, M. R. Pie, D. R. Barneche, L. G. R. Oliveira-Santos, and S. R. Floeter. 2016. Isolation drives taxonomic and functional nestedness in tropical reef fish faunas. Ecography:n/a-n/a.

Best, R. J., N. C. Caulk, J. J. Stachowicz, and J. Emmett Duffy. 2013. Trait vs. phylogenetic diversity as predictors of competition and community composition in herbivorous marine amphipods. Ecol Lett **16**:72-80.

Blomberg, S. P., T. Garland, Jr., and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution **57**:717-745.

Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical Ecology with R. Springer New York Dordrecht London Heidelberg.

Borcard, D., and P. Legendre. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecological Modelling **153**:51-68.

Borcard, D., P. Legendre, and P. Drapeau. 1992. Partialling out the Spatial Component of Ecological Variation. Ecology **73**:1045.

Boven, L., and L. Brendonck. 2009. Impact of hydroperiod on seasonal dynamics in temporary pool cladoceran communities. Fundamental and Applied Limnology / Archiv für Hydrobiologie **174**:147-157.

Braband, A., S. Richter, R. Hiesel, and G. Scholtz. 2002. Phylogenetic relationships within the Phyllopoda (Crustacea, Branchiopoda) based on mitochondrial and nuclear markers. Mol Phylogenet Evol **25**:229-244.

Brans, K. I., L. Govaert, J. Engelen, A. T. Gianuca, C. Souffreau, and L. D. Meester. 2016. Eco-evolutionary dynamics in urbanised landscapes: Evolution, species sorting and the change in zooplankton body size along urbanisation gradients.

Philosophical Transactions of the Royal Society B: Biological Sciences.

Brendonck, L., M. Jocqué, K. Tuytens, B. V. Timms, and B. Vanschoenwinkel. 2015. Hydrological stability drives both local and regional diversity patterns in rock pool metacommunities. Oikos **124**:741-749.

Brett, M. T., D. C. Müller-Navarra, and P. Sang-Kyu. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. Limnology and Oceanography **45**:1564-1575.

Brooks, J. L., and S. I. Dodson. 1965. Predation, Body Size, and Composition of Plankton. Science **150**:28-35.

Brown, B. L., E. R. Sokol, J. Skelton, and B. Tornwall. 2016. Making sense of metacommunities: dispelling the mythology of a metacommunity typology. Oecologia.

Burns, C. W. 1969. Relation between filtering rate, temperature, and body size in four species of Daphnia. Limnology and Oceanography **14**:693-700.

Buschke, F. T., L. De Meester, L. Brendonck, and B. Vanschoenwinkel. 2015. Partitioning the variation in African vertebrate distributions into environmental and spatial components - exploring the link between ecology and biogeography. Ecography **38**:450-461.

Butterfield, B. J., K. N. Suding, and S. Lavorel. 2013. Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in a complex landscape. Journal of Ecology **101**:9-17.

Cadotte, M., C. H. Albert, and S. C. Walker. 2013. The ecology of differences: assessing community assembly with trait and evolutionary distances. Ecol Lett **16**:1234-1244.

Cadotte, M. W. 2013. Experimental evidence that evolutionarily diverse assemblages result in higher productivity. Proc Natl Acad Sci U S A **110**:8996-9000.

Cadotte, M. W. 2015. Phylogenetic diversity and productivity: gauging interpretations from experiments that do not manipulate phylogenetic diversity. Functional Ecology:n/a-n/a.

Cadotte, M. W., B. J. Cardinale, and T. H. Oakley. 2008. Evolutionary history and the effect of biodiversity on plant productivity. Proc Natl Acad Sci U S A **105**:17012-17017.

Cadotte, M. W., J. Cavender-Bares, D. Tilman, and T. H. Oakley. 2009. Using Phylogenetic, Functional and Trait Diversity to

Understand Patterns of Plant Community Productivity. PLoS ONE **4**:e5695.

Cadotte, M. W., R. Dinnage, and D. Tilman. 2012. Phylogenetic diversity promotes ecosystem stability. Ecology **93**:S223-S233.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59-67.

Cardinale, B. J., D. S. Srivastava, J. Emmett Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**:989-992.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences **104**:18123-18128.

Carroll, I. T., B. J. Cardinale, and R. M. Nisbet. 2011. Niche and fitness differences relate the maintenance of diversity to ecosystem function. Ecology **92**:1157-1165.

Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. Am Nat **163**:823-843.

Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic Structure of Floridian Plant Communities Depends on Taxonomic and Spatial Scale. Ecology **87**:S109-S122.

Cavender-Bares, J., K. H. Kozak, P. V. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecol Lett **12**:693-715.

Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. Garcia, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. Science Advances **1**:e1400253-e1400253.

Chang, C.-R., M.-H. Li, and S.-D. Chang. 2007. A preliminary study on the local cool-island intensity of Taipei city parks. Landscape and Urban Planning **80**:386-395.

Chao, A., C.-H. Chiu, and T. C. Hsieh. 2012. Proposing a resolution to debates on diversity partitioning. Ecology **93**:2037-2051.

Chase, J. M., T. M. Knight, and H. Cornell. 2013. Scale-dependent effect sizes of ecological drivers on biodiversity: why standardised sampling is not enough. Ecol Lett **16**:17-26.

Concepción, E. D., M. Moretti, F. Altermatt, M. P. Nobis, and M. K. Obrist. 2015. Impacts of urbanisation on biodiversity: the role of species mobility, degree of specialisation and spatial scale. Oikos **124**:1571-1582.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecol Lett **8**:1175-1182.

Cottenie, K., and L. De Meester. 2004. Metacommunity structure: synergy of biotic interactions as seletive agents and dispersal as fuel. Ecology **85**:114-119.

Cottenie, K., E. Michels, N. Nuytten, and L. De Meester. 2003. Zooplankton Metacommunity Structure: Regional Vs. Local Processes in Highly Interconnected Ponds. Ecology **84**:991-1000.

Crist, Thomas O., Joseph A. Veech, Jon C. Gering, and Keith S. Summerville. 2003. Partitioning Species Diversity across Landscapes and Regions: A Hierarchical Analysis of α, β, and γ Diversity. Am Nat **162**:734-743.

Cyr, H., and J. M. Curtis. 1999. Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. Oecologia **118**:306-315.

D'agata, S., D. Mouillot, M. Kulbicki, S. Andréfouët, David R. Bellwood, Joshua E. Cinner, Peter F. Cowman, M. Kronen, S. Pinca, and L. Vigliola. 2014. Human-Mediated Loss of Phylogenetic and Functional Diversity in Coral Reef Fishes. Current Biology **24**:555-560.

De Bie, T., L. De Meester, L. Brendonck, K. Martens, B. Goddeeris, D. Ercken, H. Hampel, L. Denys, L. Vanhecke, K. Van der Gucht, J. Van Wichelen, W. Vyverman, and S. A. Declerck. 2012. Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. Ecol Lett **15**:740-747.

De Meester, L., S. Declerck, R. Stoks, G. Louette, F. Van De Meutter, T. De Bie, E. Michels, and L. Brendonck. 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. Aquatic Conservation: Marine and Freshwater Ecosystems **15**:715-725.

De Meester, L., J. Vanoverbeke, L. J. Kilsdonk, and M. C. Urban. 2016. Evolving Perspectives on Monopolization and Priority Effects. Trends in Ecology & Evolution **31**:136-146.

Debastiani, V. J., and L. d. S. Duarte. 2016. Evolutionary Models and Phylogenetic Signal Assessment via Mantel Test. Evolutionary Biology.

Declerck, S., T. De Bie, D. Ercken, H. Hampel, S. Schrijvers, J. Van Wichelen, V. Gillard, R. Mandiki, B. Losson, D. Bauwens, S. Keijers, W. Vyverman, B. Goddeeris, L. De meester, L. Brendonck, and K. Martens. 2006. Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. Biological Conservation **131**:523-532.

Declerck, S., J. Vandekerkhove, L. Johansson, K. Muylaert, J. M. Conde-Porcuna, K. Van der Gucht, C. Pérez-Martínez, T. Lauridsen, K. Schwenk, G. Zwart, W. Rommens, J. López-Ramos, E. Jeppesen, W. Vyverman, L. Brendonck, and L. De Meester. 2005. Multi-Group Biodiversity in Shallow Lakes Along Gradients of Phosphorus and Water Plant Cover. Ecology **86**:1905-1915.

Declerck, S., M. Vanderstukken, A. Pals, K. Muylaert, and L. D. Meester. 2007. Plankton Biodiversity Along a Gradient of Productivity and Its Mediation by Macrophytes. Ecology **88**:2199-2210.

Declerck, S. A. J., E. S. Bakker, B. van Lith, A. Kersbergen, and E. van Donk. 2011a. Effects of nutrient additions and macrophyte composition on invertebrate community assembly and diversity in experimental ponds. Basic and Applied Ecology **12**:466-475.

Declerck, S. A. J., J. S. Coronel, P. Legendre, and L. Brendonck. 2011b. Scale dependency of processes structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands. Ecography **34**:296-305.

Declerck, S. A. J., C. Winter, J. B. Shurin, C. A. Suttle, and B. Matthews. 2012. Effects of patch connectivity and heterogeneity on metacommunity structure of planktonic bacteria and viruses. The ISME Journal **7**:533-542.

DeMott, W. R. 1986. The role of taste in food selection by freshwater zooplankton. Oecologia **69**:334-340.

DeMott, W. R., and W. C. Kerfoot. 1982. Competition Among Cladocerans: Nature of the Interaction Between Bosmina and Daphnia. Ecology **63**:1949.

Deraison, H., I. Badenhausser, N. Loeuille, C. Scherber, N. Gross, and N. Haddad. 2015. Functional trait diversity across trophic levels determines herbivore impact on plant community biomass. Ecol Lett:n/a-n/a.

Díaz, S., A. Purvis, J. H. C. Cornelissen, G. M. Mace, M. J. Donoghue, R. M. Ewers, P. Jordano, and W. D. Pearse. 2013. Functional traits, the phylogeny of function, and ecosystem service vulnerability. Ecology and Evolution **3**:2958-2975.

Dodson, S. I. 1974. Zooplankton Competition and Predation: An Experimental Test of the Size-Efficiency Hypothesis. Ecology **55**:605.

Dodson, S. I., S. E. Arnott, and K. L. Cottingham. 2000. The Relationship in Lake Communities between Primary Productivity and Species Richness. Ecology **81**:2662-2679.

Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage Time Series Reveal Biodiversity Change but Not Systematic Loss. Science **344**:296-299.

Downing, A. L., B. L. Brown, and M. A. Leibold. 2014. Multiple diversity–stability mechanisms enhance population and community stability in aquatic food webs. Ecology **95**:173-184.

Dray, S., R. Pélissier, P. Couteron, M. J. Fortin, P. Legendre, P. R. Peres-Neto, E. Bellier, R. Bivand, F. G. Blanchet, M. De Cáceres, A. B. Dufour, E. Heegaard, T. Jombart, F. Munoz, J. Oksanen, J. Thioulouse, and H. H. Wagner. 2012. Community ecology in the age of multivariate multiscale spatial analysis. Ecological Monographs **82**:257-275.

Driscoll, D. A., and D. B. Lindenmayer. 2009. Empirical tests of metacommunity theory using an isolation gradient. Ecological Monographs **79**:485-501.

Duarte, L. D. S., R. S. Bergamin, V. Marcilio-Silva, G. D. D. S. Seger, and M. C. M. Marques. 2014. Phylobetadiversity among Forest Types in the Brazilian Atlantic Forest Complex. PLoS ONE **9**:e105043.

Duarte, L. D. S., P. V. Prieto, and V. D. Pillar. 2012. Assessing spatial and environmental drivers of phylogenetic structure in BrazilianAraucariaforests. Ecography **35**:952-960.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z.-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2005. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews **81**:163.

Duffy, J. E., P. L. Reynolds, C. Boström, J. A. Coyer, M. Cusson, S. Donadi, J. G. Douglass, J. S. Eklöf, A. H. Engelen, B. K. Eriksson, S. Fredriksen, L. Gamfeldt, C. Gustafsson, G. Hoarau, M. Hori, K. Hovel, K. Iken, J. S. Lefcheck, P.-O. Moksnes, M. Nakaoka, M. I. O'Connor, J. L. Olsen, J. P. Richardson, J. L. Ruesink, E. E. Sotka, J. Thormar, M. A. Whalen, J. J. Stachowicz, and B. Worm. 2015. Biodiversity mediates top-down control in eelgrass ecosystems: a global comparative-experimental approach. Ecol Lett:n/a-n/a.

Ewers, R. M., R. K. Didham, W. D. Pearse, V. Lefebvre, I. M. Rosa, J. M. Carreiras, R. M. Lucas, and D. C. Reuman. 2013. Using landscape history to predict biodiversity patterns in fragmented landscapes. Ecol Lett **16**:1221-1233.

Faith, D. P. 2015. Phylogenetic diversity, functional trait diversity and extinction: avoiding tipping points and worst-case losses. Philosophical Transactions of the Royal Society B: Biological Sciences **370**:20140011-20140011.

Fernandes, I. M., R. Henriques-Silva, J. Penha, J. Zuanon, and P. R. Peres-Neto. 2013. Spatiotemporal dynamics in a seasonal metacommunity structure is predictable: the case of floodplain-fish communities. Ecography:no-no.

Flynn, D. F. B., N. Mirotchnick, M. Jain, M. I. Palmer, and S. Naeem. 2011. Functional and phylogenetic diversity as predictors of biodiversity–ecosystem-function relationships. Ecology **92**:1573-1581.

Frishkoff, L. O., D. S. Karp, L. K. M'Gonigle, C. D. Mendenhall, J. Zook, C. Kremen, E. A. Hadly, and G. C. Daily. 2014. Loss of avian phylogenetic diversity in neotropical agricultural systems. Science **345**:1343-1346.

Fukami, T. 2015. Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annual Review of Ecology, Evolution, and Systematics **46**:1-23.

Gerhold, P., J. F. Cahill, M. Winter, I. V. Bartish, and A. Prinzing. 2015. Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). Functional Ecology:n/a-n/a.

Gianuca, A. T., S. A. J. Declerck, M. W. Cadotte, C. Souffreau, T. D. Bie, and L. D. Meester. 2016a. Integrating trait and phylogenetic distances to assess scale-dependent community assembly processes. Ecography.

Gianuca, A. T., R. A. Dias, V. J. Debastiani, and L. D. S. Duarte. 2014. Habitat filtering influences the phylogenetic structure of avian communities across a coastal gradient in southern Brazil. Austral Ecology **39**:29-38.

Gianuca, A. T., J. H. Pantel, and L. De Meester. 2016b. Disentangling the effect of body size and phylogenetic distances on zooplankton top-down control of algae. Proceedings of the Royal Society B: Biological Sciences **283**:20160487.

Gliwicz, Z. M., and W. Lampert. 1990. Food Thresholds in Daphnia Species in the Absence and Presence of Blue-Green Filaments. Ecology **71**:691.

Graham, C. H., and P. V. A. Fine. 2008. Phylogenetic beta diversity: linking ecological and evolutionary processes across space in time. Ecol Lett **11**:1265-1277.

Graham, C. H., J. L. Parra, C. Rahbek, and J. A. McGuire. 2009. Phylogenetic structure in tropical hummingbird communities. Proc Natl Acad Sci U S A **106 Suppl 2**:19673-19678.

Grainger, T. N., and B. Gilbert. 2016. Dispersal and diversity in experimental metacommunities: linking theory and practice. Oikos **125**:1213-1223.

Gravel, D., T. Bell, C. Barbera, M. Combe, T. Pommier, and N. Mouquet. 2012. Phylogenetic constraints on ecosystem functioning. Nature Communications **3**:1117.

Griffith, D. A., and P. R. Peres-Neto. 2006. Spatial Modeling in Ecology: The Flexibility of Eigenfunction Spatial Analyses. Ecology **87**:2603-2613.

Grimm, N. B., S. H. Faeth, N. E. Golubiewski, C. L. Redman, J. Wu, X. Bai, and J. M. Briggs. 2008. Global Change and the Ecology of Cities. Science **319**:756-760.

Guelzow, N., F. Muijsers, R. Ptacnik, and H. Hillebrand. 2016. Functional and structural stability are linked in phytoplankton metacommunities of different connectivity. Ecography.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**:95-98.

Hamada, S., and T. Ohta. 2010. Seasonal variations in the cooling effect of urban green areas on surrounding urban areas. Urban Forestry & Urban Greening **9**:15-24.

Hansen, B., P. K. BjØRnsen, and P. J. Hansen. 1994. The size ratio between planktonic predators and their prey. Limnology and Oceanography **39**:395-403.

Hanski, I. 1998. Metapopulation dynamics. Nature **396**:41-49.

Hargrave, C. W., K. D. Hambright, and L. J. Weider. 2011. Variation in resource consumption across a gradient of increasing intra- and interspecific richness. Ecology **92**:1226-1235.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for Light Causes Plant Biodiversity Loss After Eutrophication. Science **324**:636-638.

Hautier, Y., D. Tilman, F. Isbell, E. W. Seabloom, E. T. Borer, and P. B. Reich. 2015. Anthropogenic environmental changes affect ecosystem stability via biodiversity. Science **348**:336-340.

Hector, A., E. Bazeley-White, M. Loreau, S. Otway, and B. Schmid. 2002. Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. Ecol Lett **5**:502-511.

Helmus, M. R., W. Keller, M. J. Paterson, N. D. Yan, C. H. Cannon, and J. A. Rusak. 2010. Communities contain closely related species during ecosystem disturbance. Ecol Lett **13**:162-174.

Henriques-Silva, R., Z. Lindo, and P. R. Peres-Neto. 2013. A community of metacommunities: exploring patterns in species distributions across large geographical areas. Ecology **94**:627-639.

Hillebrand, H., and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. Ecol Lett **12**:1405-1419.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking Community Assembly through the Lens of Coexistence Theory. Annual Review of Ecology, Evolution, and Systematics **43**:227-248.

Holyoak, M., M. A. Leibold, and R. D. Holt. 2005. Metacommunities : spatial dynamics and ecological communities. University of Chicago Press, Chicago.

Hooftman, D. A. P., B. Edwards, and J. M. Bullock. 2015. Reductions in connectivity and habitat quality drive local extinctions in a plant diversity hotspot. Ecography:n/a-n/a.

Hortal, J., J. A. F. Diniz-Filho, L. M. Bini, M. Á. Rodríguez, A. Baselga, D. Nogués-Bravo, T. F. Rangel, B. A. Hawkins, and J. M. Lobo. 2011. Ice age climate, evolutionary constraints and diversity patterns of European dung beetles. Ecol Lett **14**:741-748.

Hortal, J., J. C. Nabout, J. Calatayud, F. M. Carneiro, A. Padial, A. M. C. Santos, T. Siqueira, F. Bokma, L. M. Bini, and M. Ventura. 2014. Perspectives on the use of lakes and ponds as model systems for macroecological research. Journal of Limnology **73**.

Howeth, J. G., and M. A. Leibold. 2010. Prey dispersal rate affects prey species composition and trait diversity in response to multiple predators in metacommunities. Journal of Animal Ecology **79**:1000-1011.

Jeppesen, E., B. Moss, H. Bennion, L. Carvalho, L. DeMeester, H. Feuchtmayr, N. Friberg, M. O. Gessner, M. Hefting, T. L. Lauridsen, L. Liboriussen, H. J. Malmquist, L. May, M. Meerhoff, J. S. Olafsson, M. B. Soons, and J. T. A. Verhoeven. 2010. Chapter 6: Interaction of Climate Change and Eutrophication. In: M. Kernan, R.W. Battarbee & B.R. Moss (eds). Climate change impacts on freshwater ecosystems. Wiley-Blackwell.

Johnson, P. T. J., J. T. Hoverman, V. J. McKenzie, A. R. Blaustein, K. L. D. Richgels, and M. Cadotte. 2013. Urbanization and wetland communities: applying metacommunity theory to understand the local and landscape effects. Journal of Applied Ecology **50**:34-42.

Kahmen, A., C. Renker, S. B. Unsicker, and N. Buchmann. 2006. Niche Complementarity for Nitrogen: An Explanation for the Biodiversity and Ecosystem Functioning Relationship? Ecology **87**:1244-1255.

Kaye, J., P. Groffman, N. Grimm, L. Baker, and R. Pouyat. 2006. A distinct urban biogeochemistry? Trends in Ecology & Evolution **21**:192-199.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics **26**:1463-1464.

Kim, J., and M. J. Sanderson. 2008. Penalized likelihood phylogenetic inference: bridging the parsimony-likelihood gap. Syst Biol **57**:665-674.

Kleerekoper, L., M. van Esch, and T. B. Salcedo. 2012. How to make a city climate-proof, addressing the urban heat island effect. Resources, Conservation and Recycling **64**:30-38.

Knapp, S., L. Dinsmore, C. Fissore, S. E. Hobbie, I. Jakobsdottir, J. Kattge, J. Y. King, S. Klotz, J. P. McFadden, and J. Cavender-Bares. 2012. Phylogenetic and functional characteristics of household yard floras and their changes along an urbanization gradient. Ecology **93**:S83-S98.

Knapp, S., I. Kühn, O. Schweiger, and S. Klotz. 2008. Challenging urban species diversity: contrasting phylogenetic patterns across plant functional groups in Germany. Ecol Lett **11**:1054-1064.

Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015. Plant functional traits and the multidimensional nature of species coexistence. Proceedings of the National Academy of Sciences **112**:797-802.

Kruk, C., L. Rodr� ́Guez-Gallego, M. Meerhoff, F. Quintans, G. Lacerot, N. S. Mazzeo, F. Scasso, J. C. Paggi, E. T. H. M. Peeters, and S. Marten. 2009. Determinants of biodiversity in subtropical shallow lakes (Atlantic coast, Uruguay). Freshwater Biology **54**:2628-2641.

Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology **91**:299-305.

Lampert, W., and U. Sommer. 2007. Limnoecology. 2 edition. Oxford University Press Inc.,, New York.

Larsen, T. H., N. M. Williams, and C. Kremen. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecol Lett **8**:538-547.

Lassmann, T., and E. L. L. Sonnhammer. 2006. Kalign, Kalignvu and Mumsa: web servers for multiple sequence alignment. Nucleic Acids Research **34**:W596-W599.

Lefcheck, J. S., V. A. G. Bastazini, and J. N. Griffin. 2014. Choosing and using multiple traits in functional diversity research. Environmental Conservation **42**:104-107.

Lefcheck, J. S., J. E. K. Byrnes, F. Isbell, L. Gamfeldt, J. N. Griffin, N. Eisenhauer, M. J. S. Hensel, A. Hector, B. J. Cardinale, and J. E. Duffy. 2015. Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. Nature Communications **6**:6936.

Legendre, P. 2014. Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography **23**:1324-1334.

Legendre, P., and L. Legendre. 2012. Numerical Ecology. Developments in Environmental Modelling **24**:990.

Leibold, M. A., E. P. Economo, and P. Peres-Neto. 2010. Metacommunity phylogenetics: separating the roles of environmental filters and historical biogeography. Ecol Lett **13**:1290-1299.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett **7**:601-613.

Leibold, M. A., and G. M. Mikkelson. 2002. Coherence, species turnover, and boundary clumping: elements of meta-community structure. Oikos **97**:237-250.

Leprieur, F., P. A. Tedesco, B. Hugueny, O. Beauchard, H. H. Dürr, S. Brosse, and T. Oberdorff. 2011. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. Ecol Lett **14**:325-334.

Lessard, J. P., M. K. Borregaard, J. A. Fordyce, C. Rahbek, M. D. Weiser, R. R. Dunn, and N. J. Sanders. 2011. Strong influence of regional species pools on continent-wide structuring of local communities. Proceedings of the Royal Society B: Biological Sciences **279**:266-274.

Levine, J. M., and J. HilleRisLambers. 2009. The importance of niches for the maintenance of species diversity. Nature **461**:254-257.

Li, J., C. Song, L. Cao, F. Zhu, X. Meng, and J. Wu. 2011. Impacts of landscape structure on surface urban heat islands: A case study of Shanghai, China. Remote Sensing of Environment **115**:3249-3263.

Liu, C., B. Guénard, B. Blanchard, Y.-Q. Peng, and E. P. Economo. 2016. Reorganization of taxonomic, functional, and phylogenetic ant biodiversity after conversion to rubber plantation. Ecological Monographs **86**:215-227.

Logue, J. B., N. Mouquet, H. Peter, and H. Hillebrand. 2011. Empirical approaches to metacommunities: a review and comparison with theory. Trends in Ecology & Evolution **26**:482-491.

Lopes, P. M., R. Bozelli, L. M. Bini, J. M. Santangelo, and S. A. J. Declerck. 2016. Contributions of airborne dispersal and dormant propagule recruitment to the assembly of rotifer and crustacean zooplankton communities in temporary ponds. Freshwater Biology **61**:658-669.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature **412**:72-76.

Louette, G., T. De Bie, J. Vandekerkhove, S. Declerck, and L. De Meester. 2007. Analysis of the inland cladocerans of Flanders (Belgium) –

Inferring changes over the past 70 years. Belgian Journal of Zoology **137**:117-123.

Lynch, M. 1980. The evolution of cladoceran life histories. The Quarterly Review of Biology **55**:23-42.

Maestre, F. T., J. L. Quero, N. J. Gotelli, A. Escudero, V. Ochoa, M. Delgado-Baquerizo, M. Garcia-Gomez, M. A. Bowker, S. Soliveres, C. Escolar, P. Garcia-Palacios, M. Berdugo, E. Valencia, B. Gozalo, A. Gallardo, L. Aguilera, T. Arredondo, J. Blones, B. Boeken, D. Bran, A. A. Conceicao, O. Cabrera, M. Chaieb, M. Derak, D. J. Eldridge, C. I. Espinosa, A. Florentino, J. Gaitan, M. G. Gatica, W. Ghiloufi, S. Gomez-Gonzalez, J. R. Gutierrez, R. M. Hernandez, X. Huang, E. Huber-Sannwald, M. Jankju, M. Miriti, J. Monerris, R. L. Mau, E. Morici, K. Naseri, A. Ospina, V. Polo, A. Prina, E. Pucheta, D. A. Ramirez-Collantes, R. Romao, M. Tighe, C. Torres-Diaz, J. Val, J. P. Veiga, D. Wang, and E. Zaady. 2012. Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. Science **335**:214-218.

Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecol Lett **13**:1085-1093.

McCauley, D. E., J. Raveill, and J. Antonovics. 1995. Local founding events as determinants of genetic structure in a plant metapopulation. Heredity **75**:630-636.

McGill, B., B. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology & Evolution **21**:178-185.

McKnight, M. W., P. S. White, R. I. McDonald, J. F. Lamoreux, W. Sechrest, R. S. Ridgely, and S. N. Stuart. 2007. Putting Beta-

Diversity on the Map: Broad-Scale Congruence and Coincidence in the Extremes. PLoS Biology **5**:e272.

Melo, A. S., T. F. L. V. B. Rangel, and J. A. F. Diniz-Filho. 2009. Environmental drivers of beta-diversity patterns in New-World birds and mammals. Ecography **32**:226-236.

Messier, J., B. J. McGill, and M. J. Lechowicz. 2010. How do traits vary across ecological scales? A case for trait-based ecology. Ecol Lett **13**:838-848.

Monnet, A.-C., F. Jiguet, C. N. Meynard, D. Mouillot, N. Mouquet, W. Thuiller, and V. Devictor. 2014. Asynchrony of taxonomic, functional and phylogenetic diversity in birds. Global Ecology and Biogeography **23**:780-788.

Moore, M., and C. Folt. 1993. Zooplankton body size and community structure: Effects of thermal and toxicant stress. Trends in Ecology & Evolution **8**:178-183.

Moss, B. 2010. Ecology of Freshwaters: A View for the Twenty-First Century. 4th edition. Wiley-Blackwell Publishing.

Moss, B., S. Kosten, M. Meerhoff, R. W. Battarbee, E. Jeppesen, N. s. Mazzeo, K. Havens, G. Lacerot, Z. Liu, L. D. Meester, H. Paerl, and M. Scheffer. 2011. Allied attack: climate change and eutrophication. Inland Waters **1**:101-105.

Mouquet, N., V. Devictor, C. N. Meynard, F. Munoz, L.-F. Bersier, J. Chave, P. Couteron, A. Dalecky, C. Fontaine, D. Gravel, O. J. Hardy, F. Jabot, S. Lavergne, M. Leibold, D. Mouillot, T. Münkemüller, S. Pavoine, A. Prinzing, A. S. L. Rodrigues, R. P. Rohr, E. Thébault, and W. Thuiller. 2012. Ecophylogenetics: advances and perspectives. Biological Reviews **87**:769-785.

Mouquet, N., and M. Loreau. 2003. Community Patterns in Source - Sink Metacommunities. Am Nat **162**:544-557.

Mourelatos, S., and G. Lacroix. 1990. In situ filtering rates of Cladocera: Effect of body length, temperature, and food concentration. Limnology and Oceanography **35**:1101-1111.

Naeem, S., J. E. Duffy, and E. Zavaleta. 2012. The Functions of Biological Diversity in an Age of Extinction. Science **336**:1401-1406.

Oke, T. R. 1973. City size and the urban heat island. Atmospheric Environment (1967) **7**:769-779.

Oliver, T. H., M. S. Heard, N. J. B. Isaac, D. B. Roy, D. Procter, F. Eigenbrod, R. Freckleton, A. Hector, C. D. L. Orme, O. L.

Petchey, V. Proença, D. Raffaelli, K. B. Suttle, G. M. Mace, B. Martín-López, B. A. Woodcock, and J. M. Bullock. 2015. Biodiversity and Resilience of Ecosystem Functions. Trends in Ecology & Evolution.

Pavoine, S., M. Baguette, V. M. Stevens, M. A. Leibold, C. Turlure, and M. B. Bonsall. 2014. Life history traits, but not phylogeny, drive compositional patterns in a butterfly metacommunity. Ecology **95**:3304-3313.

Peres-Neto, P. R., P. Legendre, S. Dray, and D. Borcard. 2006. Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology **87**:2614-2625.

Peres-Neto, P. R., M. A. Leibold, and S. Dray. 2012. Assessing the effects of spatial contingency and environmental filtering on metacommunity phylogenetics. Ecology **93**:S14-S30.

Perrier, C., R. Guyomard, J.-L. Bagliniere, and G. Evanno. 2011. Determinants of hierarchical genetic structure in Atlantic salmon populations: environmental factors vs. anthropogenic influences. Molecular Ecology **20**:4231-4245.

Persson, J., M. T. Brett, T. Vrede, and J. L. Ravet. 2007. Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (Daphnia) in pelagic freshwater food webs. Oikos **116**:1152-1163.

Piano, E., K. De Wolf, F. Bona, D. Bonte, D. E. Bowler, M. Isaia, L. Lens, T. Merckx, D. Mertens, M. van Kerckvoorde, L. De Meester, and F. Hendrickx. 2016. Urbanization drives community shifts towards thermophilic and dispersive species at local and landscape scales. Global Change Biology.

Pickett, S. T. A., and F. A. Bazzaz. 1978. Organization of an Assemblage of Early Successional Species on a Soil Moisture Gradient. Ecology **59**:1248-1255.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**:1246752-1246752.

Pinel-Alloul, B., A. André, P. Legendre, J. A. Cardille, K. Patalas, and A. Salki. 2013. Large-scale geographic patterns of diversity and community structure of pelagic crustacean zooplankton in Canadian lakes. Global Ecology and Biogeography **22**:784-795.

Reed, D. H., and R. Frankham. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology **17**:230-237.

Riessen, H. P., J. W. Sommerville, C. Chiappari, and D. Gustafson. 1988. ChaoborusPredation, Prey Vulnerability, and their Effect in Zooplankton Communities. Canadian Journal of Fisheries and Aquatic Sciences **45**:1912-1920.

Robin, J., A. Wezel, G. Bornette, F. Arthaud, S. Angélibert, V. Rosset, and B. Oertli. 2013. Biodiversity in eutrophicated shallow lakes: determination of tipping points and tools for monitoring. Hydrobiologia **723**:63-75.

Rolland, J., M. W. Cadotte, J. Davies, V. Devictor, S. Lavergne, N. Mouquet, S. Pavoine, A. Rodrigues, W. Thuiller, L. Turcati, M. Winter, L. Zupan, F. Jabot, and H. Morlon. 2011. Using phylogenies in conservation: new perspectives. Biology Letters **8**:692-694.

Roquet, C., W. Thuiller, and S. Lavergne. 2013. Building megaphylogenies for macroecology: taking up the challenge. Ecography **36**:13-26.

Rosenfeld, J. S. 2002. Functional redundancy in ecology and conservation. Oikos **98**:156-162.

Rosindell, J., S. P. Hubbell, and R. S. Etienne. 2011. The unified neutral theory of biodiversity and biogeography at age ten. Trends Ecol Evol **26**:340-348.

Sacherová, V., and P. D. N. Hebert. 2003. The evolutionary history of the Chydoridae (Crustacea: Cladocera). Biological Journal of the Linnean Society **79**:629-643.

Saito, V. S., J. Soininen, A. A. Fonseca-Gessner, and T. Siqueira. 2015. Dispersal traits drive the phylogenetic distance decay of similarity in Neotropical stream metacommunities. Journal of Biogeography **42**:2101-2111.

Sanchez, R., F. Serra, J. Tarraga, I. Medina, J. Carbonell, L. Pulido, A. de Maria, S. Capella-Gutierrez, J. Huerta-Cepas, T. Gabaldon, J. Dopazo, and H. Dopazo. 2011. Phylemon 2.0: a suite of web-tools for molecular evolution, phylogenetics, phylogenomics and hypotheses testing. Nucleic Acids Research **39**:W470-W474.

Scheffer, M. 2004. Ecology of Shallow Lakes. Springer Netherlands.

Segre, H., R. Ron, N. De Malach, Z. Henkin, M. Mandel, R. Kadmon, and M. Vellend. 2014. Competitive exclusion, beta diversity, and deterministic vs. stochastic drivers of community assembly. Ecol Lett **17**:1400-1408.

Shurin, J. B. 2001. Interactive Effects of Predation and Dispersal on Zooplankton Communities. Ecology **82**:3404.

Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the Hierarchical Organization of Plant Diversity. Ecology **87**:S39-S49.

Soininen, J. 2014. A quantitative analysis of species sorting across organisms and ecosystems. Ecology **95**:3284-3292.

Soininen, J. 2016. Spatial structure in ecological communities - a quantitative analysis. Oikos **125**:160-166.

Soliveres, S., F. van der Plas, P. Manning, D. Prati, M. M. Gossner, S. C. Renner, F. Alt, H. Arndt, V. Baumgartner, J. Binkenstein, K. Birkhofer, S. Blaser, N. Blüthgen, S. Boch, S. Böhm, C. Börschig, F. Buscot, T. Diekötter, J. Heinze, N. Hölzel, K. Jung, V. H. Klaus, T. Kleinebecker, S. Klemmer, J. Krauss, M. Lange, E. K. Morris, J. Müller, Y. Oelmann, J. Overmann, E. Pašalić, M. C. Rillig, H. M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, J. Sikorski, S. A. Socher, E. F. Solly, I. Sonnemann, E. Sorkau, J. Steckel, I. Steffan-Dewenter, B. Stempfhuber, M. Tschapka, M. Türke, P. C. Venter, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, V. Wolters, T. Wubet, S. Wurst, M. Fischer, and E. Allan. 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. Nature **536**:456-459.

Somers, K. A., E. S. Bernhardt, J. B. Grace, B. A. Hassett, E. B. Sudduth, S. Wang, and D. L. Urban. 2013. Streams in the urban heat island: spatial and temporal variability in temperature. Freshwater Science **32**:309-326.

Spasojevic, M. J., S. Copeland, and K. N. Suding. 2014. Using functional diversity patterns to explore metacommunity dynamics: a framework for understanding local and regional influences on community structure. Ecography **37**:939-949.

Spasojevic, M. J., and K. N. Suding. 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. Journal of Ecology **100**:652-661.

Srivastava, D. S., M. W. Cadotte, A. A. MacDonald, R. G. Marushia, and N. Mirotchnick. 2012a. Phylogenetic diversity and the functioning of ecosystems. Ecol Lett **15**:637-648.

Srivastava, D. S., M. W. Cadotte, A. A. M. MacDonald, R. G. Marushia, N. Mirotchnick, and A. Mooers. 2012b. Phylogenetic diversity and the functioning of ecosystems. Ecol Lett **15**:637-648.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**:2688-2690.

Steffen, W., J. Grinevald, P. Crutzen, and J. McNeill. 2011. The Anthropocene: conceptual and historical perspectives. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **369**:842-867.

Suding, K. N., S. Lavorel, F. S. Chapin, J. H. C. Cornelissen, S. DÍAz, E. Garnier, D. Goldberg, D. U. Hooper, S. T. Jackson, and M.-L. Navas. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology **14**:1125-1140.

Swenson, N. G. 2014. Functional Phylogenetic Ecology in R. Springer New York Heidelberg Dordrecht London.

Swenson, N. G., D. L. Erickson, X. Mi, N. A. Bourg, J. Forero-Montaña, X. Ge, R. Howe, J. K. Lake, X. Liu, K. Ma, N. Pei, J. Thompson, M. Uriarte, A. Wolf, S. J. Wright, W. Ye, J. Zhang, J. K. Zimmerman, and W. J. Kress. 2012. Phylogenetic and functional alpha and beta diversity in temperate and tropical tree communities. Ecology **93**:S112-S125.

Symons, C. C., and S. E. Arnott. 2013. Regional zooplankton dispersal provides spatial insurance for ecosystem function. Global Change Biology **19**:1610-1619.

Symons, C. C., and J. B. Shurin. 2016. Climate constrains lake community and ecosystem responses to introduced predators. Proceedings of the Royal Society B: Biological Sciences **283**:20160825.

Taleghani, M., M. Tenpierik, A. van den Dobbelsteen, and D. J. Sailor. 2014. Heat in courtyards: A validated and calibrated parametric study of heat mitigation strategies for urban courtyards in the Netherlands. Solar Energy **103**:108-124.

Thompson, P. L., T. J. Davies, and A. Gonzalez. 2015. Ecosystem Functions across Trophic Levels Are Linked to Functional and Phylogenetic Diversity. PLoS ONE **10**:e0117595.

Tillmann, U., and W. Lampert. 1984. Competitive Ability of Differently SizedDaphniaSpecies: An Experimental Test. Journal of Freshwater Ecology **2**:311-323.

Tonkin, J. D., S. Stoll, S. C. Jähnig, and P. Haase. 2016. Contrasting metacommunity structure and beta diversity in an aquatic-floodplain system. Oikos **125**:686-697.

Trisos, C. H., O. L. Petchey, and J. A. Tobias. 2014. Unraveling the Interplay of Community Assembly Processes Acting on Multiple Niche Axes across Spatial Scales. Am Nat **184**:593-608.

Tucker, C. M., and T. Fukami. 2014. Environmental variability counteracts priority effects to facilitate species coexistence: evidence from nectar microbes. Proceedings of the Royal Society B: Biological Sciences **281**:20132637-20132637.

Tuomisto, H. 2010a. A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. Ecography **33**:2-22.

Tuomisto, H. 2010b. A diversity of beta diversities: straightening up a concept gone awry. Part 2. Quantifying beta diversity and related phenomena. Ecography **33**:23-45.

Ulén, B., M. Bechmann, J. Fölster, H. P. Jarvie, and H. Tunney. 2007. Agriculture as a phosphorus source for eutrophication in the north-west European countries, Norway, Sweden, United Kingdom and Ireland: a review. Soil Use and Management **23**:5-15.

Ulrich, W., M. Almeida-Neto, and N. J. Gotelli. 2009. A consumer's guide to nestedness analysis. Oikos **118**:3-17.

Ulrich, W., and N. J. Gotelli. 2010. Null model analysis of species associations using abundance data. Ecology **91**:3384-3397.

Urban, M. C. 2004. Disturbance Heterogeneity Determines Freshwater Metacommunity Structure. Ecology **85**:2971-2978.

Urban, M. C., D. K. Skelly, D. Burchsted, W. Price, and S. Lowry. 2006. Stream communities across a rural-urban landscape gradient. Diversity <html_ent glyph="@amp;" ascii="&"/> Distributions **12**:337-350.

Vamosi, S. M., S. B. Heard, J. C. Vamosi, and C. O. Webb. 2009. Emerging patterns in the comparative analysis of phylogenetic community structure. Molecular Ecology **18**:572-592.

van der Plas, F., P. Manning, E. Allan, M. Scherer-Lorenzen, K. Verheyen, C. Wirth, M. A. Zavala, A. Hector, E. Ampoorter, L. Baeten, L. Barbaro, J. Bauhus, R. Benavides, A. Benneter, F. Berthold, D. Bonal, O. Bouriaud, H. Bruelheide, F. Bussotti, M. Carnol, B. Castagneyrol, Y. Charbonnier, D. Coomes, A. Coppi, C. C. Bastias, S. Muhie Dawud, H. De Wandeler, T. Domisch, L. Finér, A. Gessler, A. Granier, C. Grossiord, V. Guyot, S. Hättenschwiler, H. Jactel, B. Jaroszewicz, F.-X. Joly, T. Jucker, J. Koricheva, H. Milligan, S. Müller, B. Muys,

D. Nguyen, M. Pollastrini, K. Raulund-Rasmussen, F. Selvi, J. Stenlid, F. Valladares, L. Vesterdal, D. Zielínski, and M. Fischer. 2016. Jack-of-all-trades effects drive biodiversity–ecosystem multifunctionality relationships in European forests. Nature Communications **7**:11109.

Vannette, R. L., and T. Fukami. 2014. Historical contingency in species interactions: towards niche-based predictions. Ecol Lett **17**:115-124.

Venail, P., K. Gross, T. H. Oakley, A. Narwani, E. Allan, P. Flombaum, F. Isbell, J. Joshi, P. B. Reich, D. Tilman, J. van Ruijven, B. J. Cardinale, and C. Fox. 2015. Species richness, but not phylogenetic diversity, influences community biomass production and temporal stability in a re-examination of 16 grassland biodiversity studies. Functional Ecology **29**:615-626.

Venail, P. A., and M. J. Vives. 2013. Phylogenetic distance and species richness interactively affect the productivity of bacterial communities. Ecology **94**:2529-2536.

Verreydt, D., L. De Meester, E. Decaestecker, M.-J. Villena, K. Van Der Gucht, P. Vannormelingen, W. Vyverman, and S. A. J. Declerck. 2012. Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. Ecol Lett **15**:218-226.

Villéger, S., G. Grenouillet, and S. Brosse. 2013. Decomposing functional β-diversity reveals that low functional β-diversity is driven by low functional turnover in European fish assemblages. Global Ecology and Biogeography **22**:671-681.

Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New Multidimensional Functional Diversity Indices for a Multifaceted Framework in Functional Ecology. Ecology **89**:2290-2301.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: intraspecific variability in community ecology. Trends in Ecology & Evolution **27**:244-252.

Vogt, R. J., P. R. Peres-Neto, and B. E. Beisner. 2013. Using functional traits to investigate the determinants of crustacean zooplankton community structure. Oikos **122**:1700-1709.

Walseng, B., D. O. Hessen, G. Halvorsen, and A. K. Schartau. 2006. Major contribution from littoral crustaceans to zooplankton species richness in lakes. Limnology and Oceanography **51**:2600-2606.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics **24**:2098-2100.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and Community Ecology. Annual Review of Ecology and Systematics **33**:475-505.

Weinstein, B. G., B. Tinoco, J. L. Parra, L. M. Brown, J. A. McGuire, F. G. Stiles, and C. H. Graham. 2014. Taxonomic, phylogenetic, and trait Beta diversity in South American hummingbirds. Am Nat **184**:211-224.

Whittaker, J. 1984. Model Interpretation from the Additive Elements of the Likelihood Function. Applied Statistics **33**:52.

Whittaker, R. H. 1960. Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs **30**:279.

Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V. Cornell, E. I. Damschen, T. Jonathan Davies, J. A. Grytnes, S. P. Harrison, B. A. Hawkins, R. D. Holt, C. M. McCain, and P. R. Stephens. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett **13**:1310-1324.

Winfree, R., J. W. Fox, N. M. Williams, J. R. Reilly, D. P. Cariveau, and K. Shea. 2015. Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. Ecol Lett:n/a-n/a.

Withers, P. J. A., and P. M. Haygarth. 2007. Agriculture, phosphorus and eutrophication: a European perspective. Soil Use and Management **23**:1-4.

Wu, J., S. Wurst, and X. Zhang. 2016. Plant functional trait diversity regulates the nonlinear response of productivity to regional climate change in Tibetan alpine grasslands. Scientific Reports **6**:35649.

Ye, L., C.-Y. Chang, C. García-Comas, G.-C. Gong, C.-h. Hsieh, and A. Beckerman. 2013. Increasing zooplankton size diversity enhances the strength of top-down control on phytoplankton through diet niche partitioning. Journal of Animal Ecology **82**:1052-1061.

Young, H. S., D. J. McCauley, M. Galetti, and R. Dirzo. 2016. Patterns, Causes, and Consequences of Anthropocene Defaunation. Annual Review of Ecology, Evolution, and Systematics **47**:333-358.

Zalasiewicz, J., M. Williams, A. Haywood, and M. Ellis. 2011. The Anthropocene: a new epoch of geological time? Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **369**:835-841.

FACULTY OF SCIENCE
DEPARTMENT OF Biology
Laboratory of Aquatic Ecology, Evolution and Conservation
Charles Deberiotstraat 32
B-3000 LEUVEN, BELGIUM
tel. + 32 16 32 37 17

andros.taroucogianuca@bio.kuleuven.be